MICROBIAL LOAD MONITOR

NASA/JSC

MCDONNELL DOUGLAS ASTRONAUTICS COMPANY - ST. LOUIS

MCDONNELL DOUGLAS CORPORATION

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MICROBIAL LOAD MONITOR FINAL REPORT

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1.0 INTRODUCTION

The Microbial Load Monitor (MLM) is an automated and computerized system for detection and identification of microorganisms. Additionally, the system is designed to enumerate and provide antimicrobic susceptibility profiles for medically significant bacteria. The system is designed to accomplish these tasks in a time of 13 hours or less versus the traditional time of 24 hours for negatives and 72 hours or more for positives usually required for standard microbiological analysis. The MLM concept differs from other methods of microbial detection in that the system is designed to accept raw untreated clinical samples and to selectively identify each group or species that may be present in a polymicrobic sample.

In order to accomplish 13-hour identification, enumeration and antimicrobial susceptibility testing from direct specimen inoculation, a series of developmental events were accomplished. Various selective-enrichment media were developed, each capable of enabling the detection of a specific species or group of microorganisms while inhibiting growth of competing groups. These media were utilized in a micro-cuvette system (Card) to enhance rapid detection and identification. This micro-culture approach was combined with solid state electro-optical techniques utilizing light emitting diodes (LED) and computer software to provide an automated monitoring and reporting device.

The system's selective microculture concept utilizes a series of freeze-dried media inside a plastic card containing small wells. Two types of cards were developed, one for clinical tests and the other for environmental tests. The Clinical Cards are inoculated directly with raw clinical material from human throats, urine, or fecal specimens while the Environmental Cards accept liquid or swab environmental samples. Careful use of chemicals, antibiotics, and chemical inhibitors plus the proper growth nutrients and substrates coax a specific metabolic activity from the desired microbes, which then precipitates an event, or series of events, which can be detected by the MLM. The time required for detection of a specific group varies from one or two hours to several hours depending upon the microorganism. Average overall detection time for the MLM is less than 8 hours. As currently developed, the MLM has the capacity to accept up to 5 clinical or 15 environmental samples at one time. At completion of a clinical sample analysis by the MLM, the following information is provided: (a) pathogen identification, (b) numbers present expressed

as $<10^5$/ml or $\geq 10^5$/ml, and (c) their antibiotic sensitivity and resistance to four selected agents is known. An environmental sample analyzed by the MLM provides pathogen identification.

The MLM concept began as a company project in 1966. A combination of company and NASA funds has permitted uninterrupted progress to the present time. The two previous NASA contracts proved concept feasibility (NAS 9-8329), and demonstrated a diagnostic capability of the MLM (NAS 9-10516).

The present contract (NAS 9-11877) began in June 1971 and had a primary task of demonstrating the reliability of the MLM as a diagnostic tool. A contract change order in February 1972 directed that the MLM shall have a high volume capability, with a capacity of up to 150 clinical samples per day. This high volume capability was a modification of the instrument used at the start of reliability testing. This modified instrument yielded a large volume of data in demonstrating the reliability of the MLM as a diagnostic tool and in addition showed what could be expected if an MLM instrument was adapted for large volume earth application use. A subsequent contract change order directed efforts toward the present system designed for the amount and type of use expected in space but also could be used to analyze earth samples. This unit with some modification could be used in the Spacelab.

High volume clinical capability was first proven under the 1972 contract change order. This concept has been applied to the McDonnell Douglas AutoMicrobic System developed under patent waivers granted to MDC by NASA. The AutoMicrobic System is now being marketed as a commercially available automated microbiological system for clinical laboratory use.

Section 2, this report, contains a historical summary of work performed under the present contract. Section 3 includes materials and methods while Section 4 presents results of MLM testing. Section 5 contains a discussion of the biological and engineering problems associated with the performance of the contract tasks. Conclusions and future recommendations are presented in Section 6.

It should be noted that references to the MLM or any elements of the MLM system in relation to the AMS or any elements of the AMS system such as AMS-MLM, AMS/MLM, or in any other form is intended to suggest or imply only that AMS proprietary technology owned by MDC was or may have been used to support work performed under this contract.

2.0 EXECUTIVE SUMMARY

This section is the executive summary of Contract NAS9-11877 which was initiated in June 1971 and has continued to the present date. That contract began after five years of work had been performed, therefore, to place the present contracted activity in historical perspective, it is worthwhile to describe briefly the origin of the idea of the Microbial Load Monitor (MLM), how that idea evolved, and how the means of implementation changed. That description will show how we arrived at technology with which the present contracted activity began. This section is written in non-technical language for management reviewers, and it contains some brief explanatory digressions in order to give understanding to that audience.

In mid-1966, microbiologists from McDonnell Douglas Astronautics Company - St. Louis (MDAC-St. Louis) visited with scientists in the Life Sciences Directorate at Johnson Spacecraft Center (JSC) to describe the MDAC-St. Louis microbiological capabilities and to learn the microbiological requirements visualized for future manned space flight. At that time several studies had been performed in ground-based, manned spacecraft simulators. These studies showed a striking change in the relative numbers of different kinds of microorganisms. Those results were of scientific interest and the JSC scientists were interested in performing similar, ecological studies of the microflora in flight. To perform those studies they had a requirement for a black box which would automatically identify and enumerate microbes without requiring the assistance of a skilled microbiologist. The source of the microbes would be the gaseous environment, hardware surfaces and the crew. Parenthetically, there was no stated or implied requirement for clinical microbiology to be used for diagnostic and therapeutic purposes. Although this requirement was valid, the JSC scientists were not willing to recommend financial support for work leading to such a device until someone was able to demonstrate proof of principle.

With that definition of requirements and constraints, MDAC microbiologists and engineers began an examination of alternate approaches that might satisfy the requirements. Three key decisions were made during that period of mental ferment which made the present MLM possible. Two of those decisions required a break with

microbiological traditions and one stuck with tradition. Those three decisions were drivers for the next 13 years of work by many people and, therefore, deserve some elaboration.

Traditional methods in a microbiological laboratory call for a standard sequence. The source sample contains a mixture of different microorganisms. The first step calls for a separation of the mixture into colonies of pure cultures of the individual types of microorganisms. The goal of the second step is to identify the nature of the microorganism in each of the different kinds of pure colonies that were isolated in the first step. The identification process is multifaceted. The visual characteristics of the colony provide initial clues. Samples are stained and observed under a microscope for additional clues. Biochemical tests may be performed and the pure colony is placed on selective media which either permits only one kind of organism to grow or which permits a visual change to occur that only one kind of organism will cause. The whole process is systematic, slow, visual and highly judgmental, but, when performed by well trained and experienced microbiologists, the results are good.

The first key decision was to by-pass the first traditional step and to identify each microorganism in mixed cultures. The next key decision was to use selective media for the identification. The final decision was to use solid state light emitting diodes (LED) and sensors to detect a change in the optical density of the selective media when growth occurred and to feed the electrical signal into electronic intelligence to determine when the change is enough to justify a call of positive identification. This latter decision was in essence to substitute electronic hardware for the eye and the judgment of the skilled microbiologist. The next stage in the development of the MLM was to implement those decisions to determine if they were the correct ones. Now 13 years later, and a tremendous amount of work, change and rework, we can say that those decisions were correct. The remainder of this section is a brief description of the work, change and rework that led to the present flight MLM configuration.

The MDAC-St. Louis team worked for almost two years, out of company funds, before it had developed the selective media and hardware technology and to fabricate a model that was capable of identifying two microorganisms in the presence

of many other kinds of microorganisms. It made the identification in two to six hours as compared with 24 to 48 hours by traditional procedures. That model was taken to JSC to demonstrate its principles in action. Figure 2-1 is a photograph of that feasibility model. It is crude by today's standards, but it incorporated

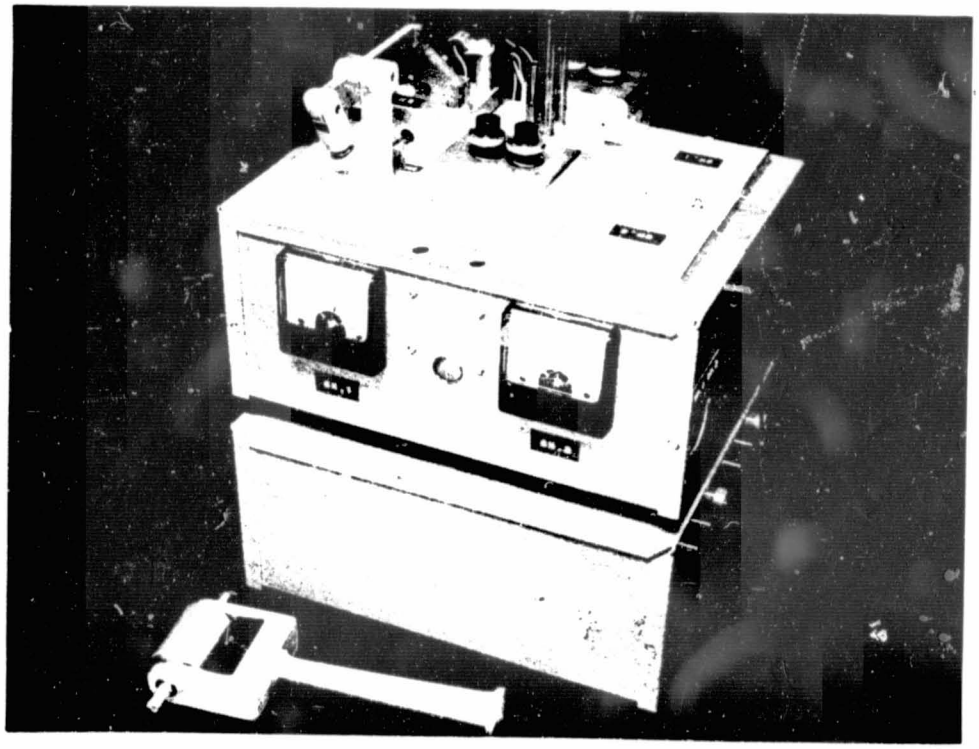

FIGURE 2-1
TWO CHANNEL MLM FEASIBILITY MODEL

the key design features described above. The selective media was in a gelatinous form stored in a dimple between two layers of plastic tape, which can be seen hanging over the right edge of the model. After an air sample of microorganisms was impacted in a hole in the top layer of tape, a third layer was placed over the two-tape assembly and a hand held roller was run over all three layers. Pressure from the roller forced the media into the sample hole. The LED looked through the sample hole to the sensor on the opposite side of the tape. The LED housing served as the incubator to maintain media temperature at 37°C. The sensor signal was fed into analog circuitry to activate the volt meters associated with each channel. As the optical density of media changed, with the growth and metabolic activity of the selected microorganism, the change in signal was hand recorded.

At that time we thought that the rate of change of signal would be primarily a function of the number of organisms in the sample, thus giving a means of enumerating the microorganisms in the sample. That simple idea for enumeration did not stand the test of time. The only LED commercially available at that time emitted light in the near infrared range. As other LEDs became available, one operating in the red range, 665 nm, was found to be more generally useful. The analog circuitry was later changed to digital. The tape method for storing media, a take-off of the Polaroid film of that day, did not turn out to be the best means of concept implementation either. However, the key principles worked.

The demonstration was met with enthusiasm on the part of the JSC scientists and in mid-1968 Contract NAS9-8329 was awarded, and Dr. Kelton Ferguson was made the JSC technical monitor - a post he has held to the present. The contract was for support of additional media development and for the design and fabrication of an engineering model capable of identifying four organisms simultaneously with a direct recording readout. Figure 2-2 is a photograph of the product. It was during the course of this contract that we realized that another approach to enumeration was in order, but the development of in vitro selective media made good progress. MDAC was awarded a second one year contract, NAS9-10516, in 1970. Included in the Statement of Work was the task, "Initiate and Perform Laboratory Studies to Develop Methods and Procedures Required for the Identification of Medically Important Microorganisms with the MLM." It was at that point that the basic MLM requirement began to change from ecological studies toward clinical application. However, MDAC was to continue to try to develop other means for the enumeration required for the ecological studies. The principle of serial dilution of the sample was used as another approach to enumeration. The idea was to sequentially dilute the sample ten-fold at each step until there was no detectable growth. Several hardware concepts were implemented to try to reduce that principle to practice. The resulting systems were cumbersome and, on the whole, unsatisfactory. However, that trial and error process had the benefit of driving the design of the media and sample containment device away from the three layered tape to a ridged plastic device. Besides not being readily adaptable to serial dilution, the plastic tape concept proved to have at least two additional major disadvantages. Forcing the gelatinous media into the sample well sometimes resulted in trapped air bubbles which the sensor read as positive whether there was growth or not. Additionally, the gelatinous media contained enough water to cause some of

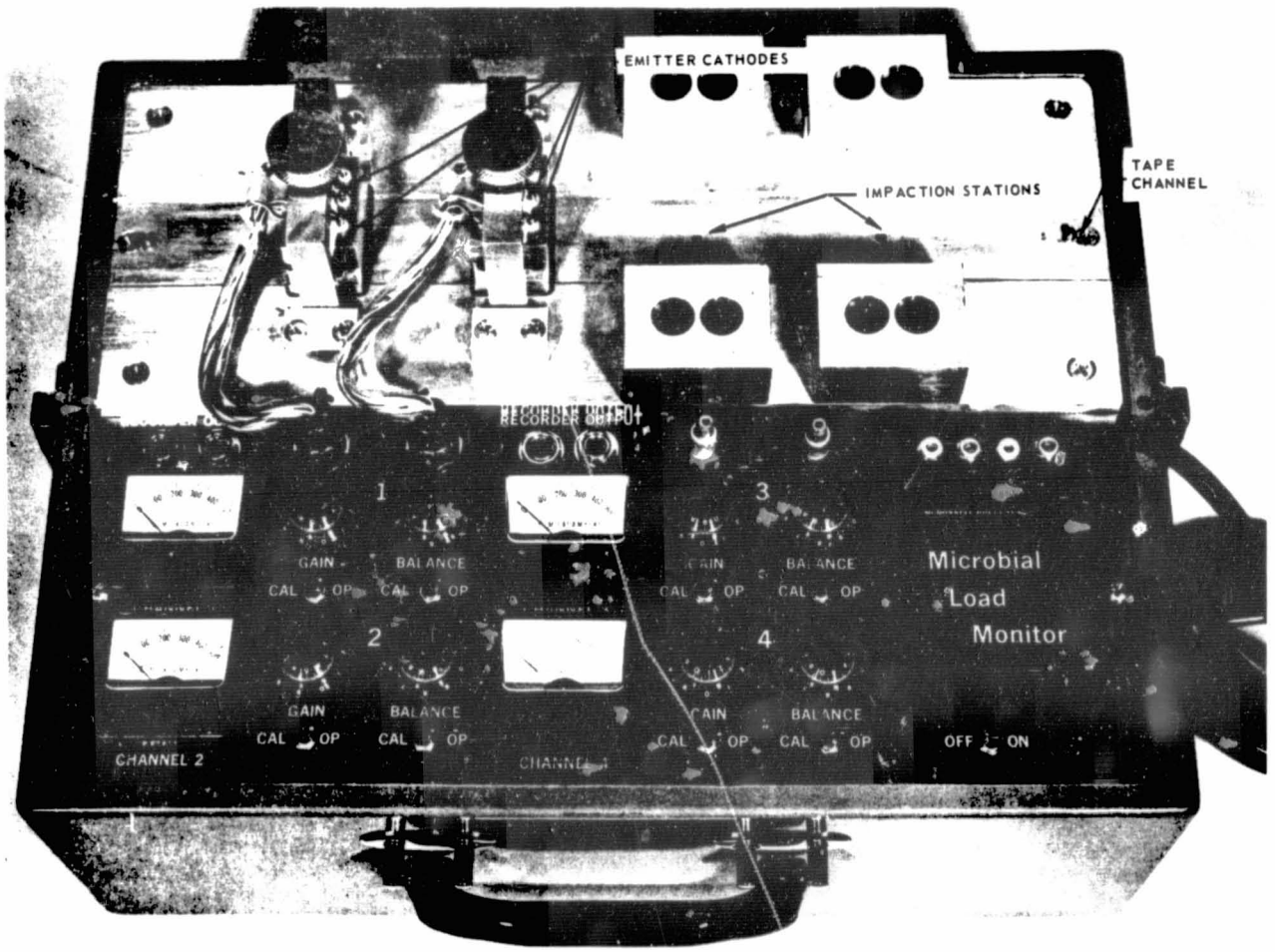

FIGURE 2-2
FOUR CHANNEL MLM

the ingredients in the media to slowly break down, thus preventing long term storage. The storage problem led to the development of solid media that was contained in the rigid plastic device that came to be known as a cassette. Figure 2-3 is a photograph of one of the early cassettes.

In addition, during the course of that contracted activity, progress on selective media for pathogenic (disease causing) microorganisms showed great promise. We began to take samples from patients in a large general hospital. We compared the results obtained with our selective media with those obtained by the hospital's clinical laboratory. The comparison was gratifying.

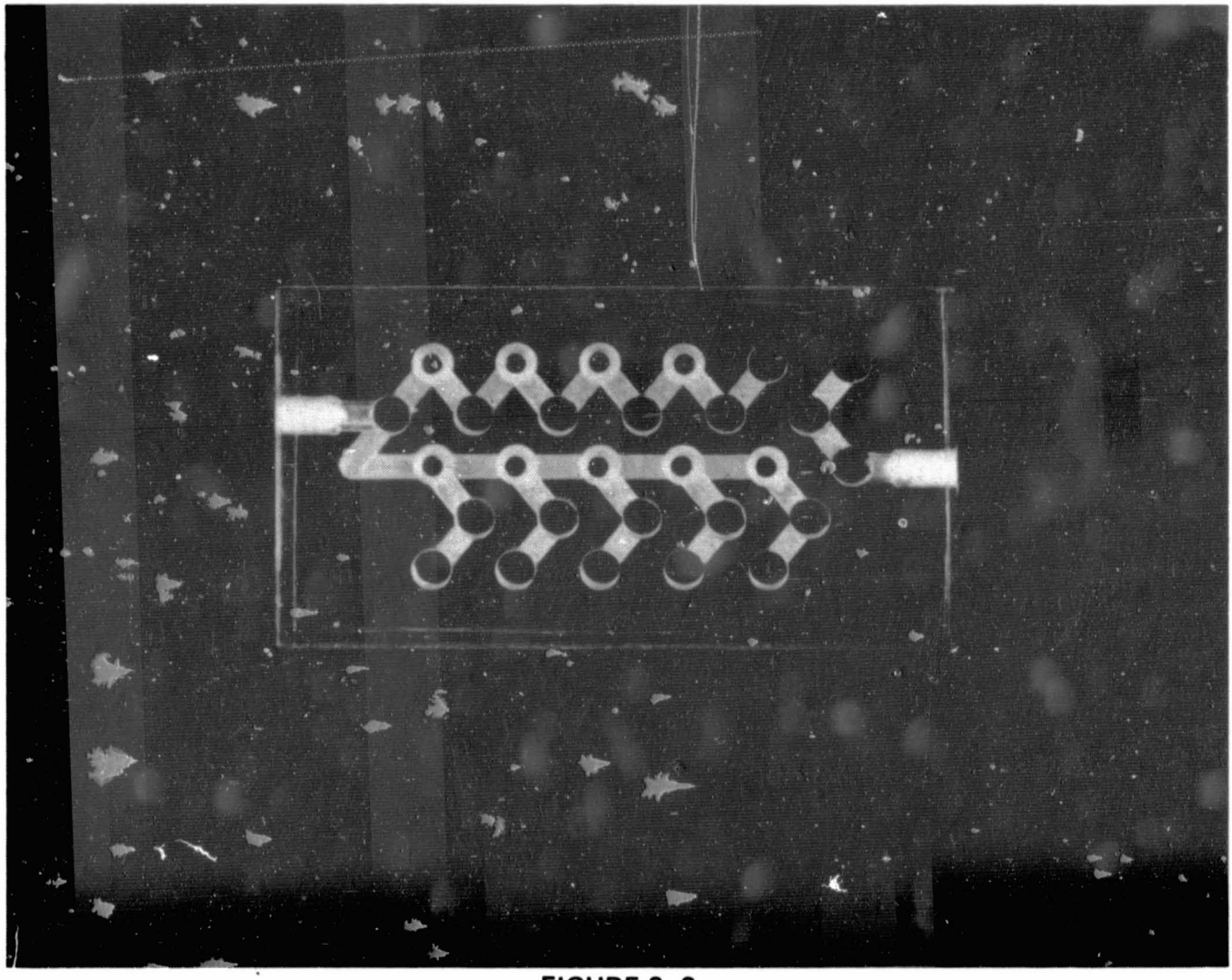

FIGURE 2–3
A MICROORGANISM CASSETTE

The LED was changed to the red range (665 nm) which, when used in conjunction with another kind of media ingredient, was helpful in reducing the time required to identify some of the slower growing microorganisms. This type of ingredient was called a chemical gain indicator. The chemical gain indicators do not enter into any kind of chemical reaction with the microbes, but as the microbes metabolize, their products produce a color change in the chemical gain indicators that is visible at 665 nm. The final change that was made to the MLM during that contract was to convert the circuitry from analog to digital. The engineering model of that device is shown in Figure 2-4. To operate the system the mixed microbial

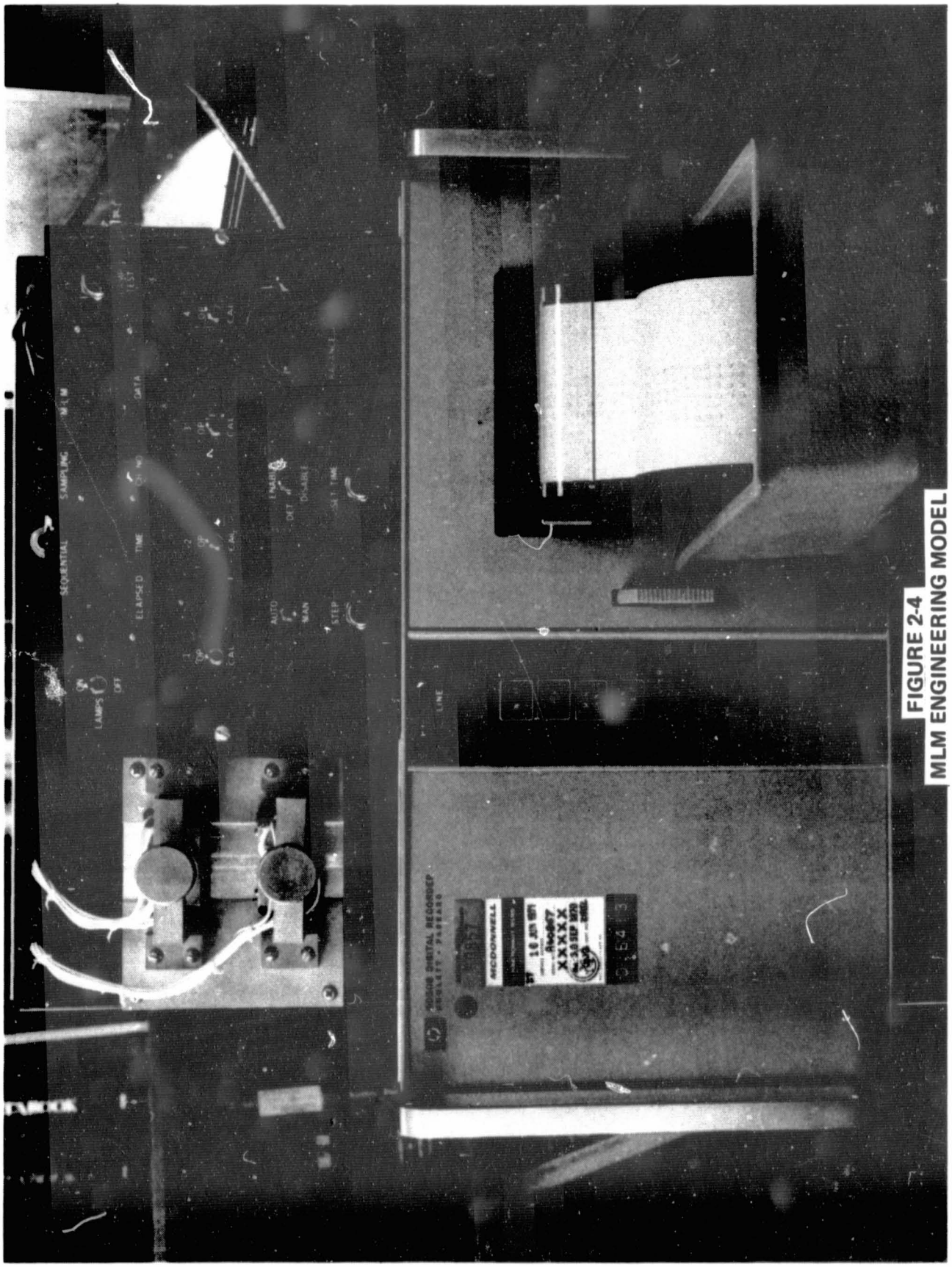

FIGURE 2-4
MLM ENGINEERING MODEL

sample was placed in sterile water. The cassette, loaded with media, was evacuated by a vacuum pump. After evacuation a septum in the cassette was perforated by a needle that was open to the water sample. This caused the sample to be loaded into the cassette. The cassette was placed in the clam-like reading head. The head controlled the temperature and the LED array sequentially looked through each cassette well and recorded time and optical density.

The developments described above were the background to the present contract. A summary of the technology status seems in order before launching into the narrative of the progress made in the present contract. The principle of using selective media to identify microorganisms in a mixed culture was workable. The media had to be stored in a solid state. The container of the media was a cassette. The sample and water for the media were loaded into the cassette by vacuum. The 665 nm LED was sensitive to microbial metabolism when aided by chemical gain indicators. The diode pair sampling was controlled by digital circuits which also drove a recorder for direct printout of results. There was still no efficient means of enumerating microbes. The whole system seemed to have application to diagnosis of certain infectious diseases.

During the time span in which the above activity was going on, Dr. Kelton Ferguson, the JSC Technical Monitor for the MDAC series of contracts, was performing clinical microbiological studies on the Apollo astronauts after they returned to earth. In those studies he found enough microbial contamination and minor infections in the astronauts to make him feel that there probably will be some instances in the future in which astronauts will incur serious infections. It looked like the MLM could grow to be an aid in identification of the infecting agent, thus permitting administration of antibiotic therapy. However, a given kind of microorganism is not always sensitive to the same antibiotic. The question arose, "How is the appropriate antibiotic to be selected?"

For the general reader, a digression is necessary to give meaning to Dr. Ferguson's burgeoning requirement. Let us assume a physician has hospitalized a patient who has the signs and symptoms of a urinary infection. The physician will ask that the patient provide a midstream urine specimen in a sterile container and will order the clinical laboratory to perform enumeration, culture, and sensitivity. He wants "enumeration" because a urine specimen nearly always has

microorganisms and their presence is clinically significant only if they are present in the urine in concentrations of 100,000 per ml or greater. This fact becomes important in the later developments of the MLM. He wants the "culture" because the process stimulated by that order will identify the pathogenic organism. That process was described earlier. He wants "sensitivity" because he wants to know what antibiotics that particular organism is resistant to and sensitive to. After the urine sample is sent, the physician may begin antibiotic treatment. He begins by playing the odds. If the patient has a significant urinary tract infection, the infecting agent is most likely to be Escherichia coli. Therefore, he orders treatment with ampicillin, an agent that may be effective against that organism. Twenty-four hours later the report from the laboratory shows that there is indeed a significant urinary tract infection and that the infecting agent is probably Pseudomonas aeruginosa - not E. coli - and the physician sees no clinical improvement in his patient. Ampicillin is not effective against Pseudomonas aeruginosa; therefore, therapy is switched to gentamicin which may be effective.

Now we return to the laboratory to get a feel for what has been going on there with respect to this patient. The original sample was diluted and that diluted sample was spread over a solid, gel medium that permits the growth of all kinds of microbes and that plate was incubated. The number of colonies that grew on this medium were counted and this gives the data necessary to determine the number of organisms in the original sample - the enumeration. At the same time the enumeration plate was prepared, a sample was thinly spread on other selective media plates to separate the individual organisms into pure culture colonies. And 24 hours from the collection of the sample the laboratory had made a tentative identification of the infecting agent. The pure cultures were subjected to definite tests to confirm or modify the tentative identification - that confirmation will therefore not be completed until 48 hours after the sample was collected. To determine the antibiotic sensitivity of the organism, a pure culture is also spread over a plate with solid media and on top of the inoculated plate 10 to 15 discs were dropped. Each of the discs contain a different antibiotic to which the microorganism may be sensitive. That plate is incubated for 18-24 hours also. At the end of that incubation period, the microbiologist observes around which discs the organism grew - insensitive - and around which discs the organism did not grow - sensitive.

From the laboratory work, described above, the physician receives a report that the organism really is Pseudomonas aeroginosa but that it is not sensitive to gentamicin. The report identifies what antibiotics the organism is sensitive to and the physician now switches to one of those.

Now we come to the Request for Proposal (RFP) to the current contract. Dr. Ferguson included in his RFP the requirement that in addition to enumeration and identification we should develop means of simultaneously determining antibiotic sensitivity of the organisms identified. The MDAC microbiologists were surprised by this additional requirement and really did not think the approach would work. Dr. Ferguson was right. It was possible to add antibiotics to selective media and simultaneously perform sensitivity tests in mixed cultures.

The present contract was started in June of 1971 and has undergone six formal contract changes through the date of this final report. Table 2-1 is a matrix of contract modifications versus categories of tasks to give an overall view of the tasks to be performed by contract period. The dates indicated on the contract modification portion of the matrix are the start dates for the modification. The statement made in the various categories of tasks are not the complete statements of work, but are intended to represent the major trends of the work to be performed. Highlights of the work performed in response to those tasks is included in the text.

The major thrusts of Exhibit A activity were: (1) to develop and test antibiotic sensitivity media, (2) to fabricate cassettes with filter beds for another approach to enumeration, and (3) to fabricate a 36-channel instrument. As alluded to above, the development of the antibiotic media progressed well for those organisms for which effective identification media had been developed. Enumeration continued to be an unsolved problem. The approach taken was one of serially reducing the concentration of microorganisms rather than serially diluting the organism by sequential addition of fluid. The idea was to place a series of small filters in the cassettes arranged so that the sample would flow through one filter into a diode viewing port and through the next filter into another viewing port and so on to signal extinction. Filters were found which would give approximately a one log reduction through each filter. However, reliable results were obtained

TABLE 2–1
CONTRACT STATEMENT OF WORK SUMMARY

CONTRACT MOD. / TASK	EXHIBIT A		EXHIBIT B 1 DEC. 1972	
	INITIAL – 21 JUNE 1971	CONT. CHANGE #1 1 FEB. 1972		
MEDIA IDENTIFICATION	CONTINUE DEVELOPMENT, REFINEMENT AND TESTING TO IMPROVE IDENTIFICATION CAPABILITY WITH SAMPLE FROM SKIN, THROAT, URINE FECES AND ENVIRONMENTAL SURFACES.	NO CHANGE	INITIATE LABORATORY SCREENING TESTS FOR DEVELOPMENT OF SELECTIVE MEDIA FOR SHIGELLA SPECIES AND NEISSERIA GONORRHOEAE	AUTOMICR FUNDING M SEE TEXT.
ANTIBIOTIC SENSITIVITY	BEGIN DEVELOPING MEDIA FOR THE DETERMINATION OF THE SENSITIVITY OF MEDICALLY IMPORTANT BACTERIA FOR WHICH THE CAPABILITY FOR IDENTIFICATION IS APPARENTLY AVAILABLE, COMPARE THE RESULTS OBTAINED WITH THE SELECTIVE MEDIA DEVELOPED TO RESULTS OBTAINED BY TRADITIONAL PROCEDURES. PERFORM THIS TASK IN TEST TUBES.	NO CHANGE	INTEGRATE AND TEST MLM CASSETTE ANTIBIOTIC SENSITIVITY CAPABILITY FOR THOSE TEST CONCEPTS DEVELOPED IN EXHIBIT A.	AUTOMICR FUNDING I SEE TEXT.
MEDIA CONTAINMENT DEVICE	INTEGRATE IDENTIFICATION (SELECTIVE) MEDIA IN CASSETTES AND BEGIN MLM TESTING FROM THREE BODY SITES.	NO CHANGE	INTEGRATE AND TEST THE MLM CASSETTE ANTIBIOTIC SENSITIVITY CAPABILITY. PROVIDES DETAILED DESIGN FOR AN INTEGRATED CASSETTE AND RETAINING DEVICE (CARD). FABRICATE AND TEST CARD TO EVALUATE DESIGN. MUST HAVE CAPABILITY OF AUTOMATED LOADING OF MEDIA.	FABRICATE CAL CARDS MLM.
STORAGE CAPABILITY	DEVELOP TECHNIQUES FOR RETURNING LIVING ORGANISMS FROM SPACE AFTER THEY HAD BEEN IDENTIFIED BY THE MLM IN SPACE.	NO CHANGE	CARD WITH MEDIA MUST BE CAPABLE OF LONG TERM STORAGE.	N
SAMPLE LOADING SYSTEM			FABRICATE, TEST, AND EVALUATE THE CLINICAL SAMPLE LOADING SYSTEM	DESIGN SAMPLE
INSTRUMENT (READER, INCUBATOR, COMPUTER AND PRINTER)	FABRICATE 36-CHANNEL SEMI–AUTOMATED SEQUENTIAL TEST MODEL OF THE MLM	STOP 36-CHANNEL. DESIGN AND FABRICATE TEST MODEL OF INSTRUMENT CAPABLE OF HANDLING 150 CASSETTES. PERFORM ENGINEERING EVALUATION.	NO ACTIVITY	DESIGN FL THAT HAS CONTROL TIC READI ONE READ PROCESSIN FIVE.
SUPPORT EQUIPMENT MEDIA LOADER CARD TAPING DEVICE	NO ACTIVITY	NO ACTIVITY	DESIGN DEVICE FOR LOADING CARDS WITH SELECTIVE MEDIA AND ANTIBIOTIC SENSITIVITY MEDIA.	COMPLETE

FOLDOUT FRAME 1

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979

B 972	EXHIBIT C 1 JAN 1974	EXHIBIT D 2 MAY 1975	EXHIBIT E 17 FEB. 1976	EXHIBIT F 15 JUNE 1977
TORY SCREEN- VELOPMENT OF FOR SHIGELLA SERIA	AUTOMICROBIC SYSTEM BEGAN FUNDING MEDIA DEVELOPMENT. SEE TEXT.	NO CHANGE	NO CHANGE	CONFIDENCE LEVELS SHALL BE ESTABLISHED BY COMPARATIVE TESTS WITH THE AMS DATA.
EST MLM TIC SENSITIVITY HOSE TEST CON- IN EXHIBIT A.	AUTOMICROBIC SYSTEM BEGAN FUNDING MEDIA DEVELOPMENT. SEE TEXT.	NO CHANGE	NO CHANGE	CONFIDENCE LEVELS SHALL BE ESTABLISHED BY COMPARATIVE TESTS WITH THE AMS DATA.
EST THE MLM TIC SENSITIVITY D DESIGN FOR ASSETTE AND (CARD). FAB- CARD TO EVALU- HAVE CAPA- TED LOADING	FABRICATE 10 PROTOTYPE CLINICAL CARDS,FOR EVALUATION IN MLM.	FABRICATE INJECTION MOLD FOR PRODUCTION OF CLINICAL AND ENVIRONMENTAL CARDS. MOLD 700 CLINICAL CARDS	CONTINUE TESTING	DELIVER 300 CLINICAL CARDS AND 50 ENVIRONMENTAL CARDS.
MUST BE CAPA- M STORAGE.	NO ACTIVITY	NO ACTIVITY	NO ACTIVITY	DETERMINE SHELF LIFE OF CARDS THAT ARE LOADED WITH MEDIA.
ND EVALUATE PLE LOADING	DESIGN FLIGHT VERSION OF SAMPLE LOADING SYSTEM.	FABRICATE INJECTION MOLDS AND PRODUCE 1400 SAMPLE RECEIVING AND CARD LOADING DEVICES (SRCLD). ASSEMBLE AS NEEDED FOR TESTING.	TEST SRCLDS WITH SWABS AND FECAL SAMPLES FABRICATE SAMPLE LOADING SYSTEM AND INCORPORATE INTO MLM PROTOTYPE.	PROVIDE SOFTWARE TO CONTROL SAMPLE LOADING SYSTEM. DELIVER 750 SRCLDS.
ITY	DESIGN FLIGHT VERSION OF MLM THAT HAS IMBEDDED COMPUTER CONTROL AND WITH 5-CARD, STATIC READING HEADS. FABRICATE ONE READING HEAD WITH SIGNAL PROCESSING CAPABILITY FOR FIVE.	FABRICATE THE KEYBOARD AND ALPHA NUMERIC DISPLAY. START SOFTWARE TO MAKE ABOVE OPERATIONAL AND FOR CONTROL OF SAMPLE FILLING SYSTEM.	FABRICATE TWO ADDITIONAL READING HEADS AND INCORPORATE INTO MLM. MODIFY MEMORY SYSTEM TO PREVENT LOSS OF MEMORY WITH POWER INTERCEPTION.	FABRICATE AND INCORPORATE FINAL TWO READING HEADS. PROVIDE SOFTWARE FOR MONITORING AND CONTROLLING SIGNALS FROM 60 CHANNELS OF EACH OF THE FIVE READING HEADS. DELIVER INSTRUMENT.
OR LOADING CTIVE MEDIA SENSITIVITY	COMPLETE EXHIBIT B ACTIVITY.	NO ACTIVITY	NO ACTIVITY	FABRICATE CARD LOADING DEVICE. FABRICATE CARD TAPING DEVICE.

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2 FOLDOUT FRAME

only when the entering microorganisms were of one kind. The results showed the principle was not effective when mixed cultures were used - probably because of the different size and shapes of the various kinds of microbes. This approach was finally abandoned at the end of the Exhibit B activity.

The 36-channel instrument specified in the Statement of Work was to be the minimal capability. MDAC instead fabricated an instrument that was designed to process 10 cassettes of 10 channels each (100 channels) simultaneously. That instrument was called the MLM-S and is shown in Figure 2-5. The cassettes were held in static reading heads which also served as incubators. A contract change to the Exhibit A was initiated about seven months into the initial contract. That change was for the purpose of making a high volume instrument (MLM-EA), that would possibly be used as a part of a larger health care system to be used on an Indian Reservation. This instrument is shown in Figure 2-6. The electronics of the MLM-S were used for the reading sequence. The incubator and cassette holder was patterned after the Kodak carousel projector. One hundred fifty cassettes were loaded into a carousel that was enclosed to permit the maintenance of the required 37°C temperature. Figure 2-6 is a photograph of that system with the Wang programmable calculator being used to monitor and plot the data generated by the system. The MLM-EA worked well but the carousel arrangement required gravity and was a dead end design for space application. However, the device proved very useful as a test bed for those media that had proved successful in test tubes to determine their suitability for use in the MLM system. Until that system was operational the volume of samples that could be tested with the full-up MLM was very small. The outcome of this more extensive testing will be amplified later.

During the activity of Exhibit B, we began testing the antibiotic sensitivity media in cassettes processed by the MLM-EA. The media tested was that developed in test tubes in the previous contract. In addition, we designed an Integrated Cassette and Retaining Device (Card). That clumsy name came from an earlier concept which visualized connecting 10 individual cassettes to a fixed manifold for loading each of the 10 with the sample simultaneously. It was planned that each cassette would enumerate and identify one kind of organism. At this point, however, the emphasis was on identification and antibiotic sensitivity and it seemed more efficient to have one rigid plastic Card identify ten different kinds

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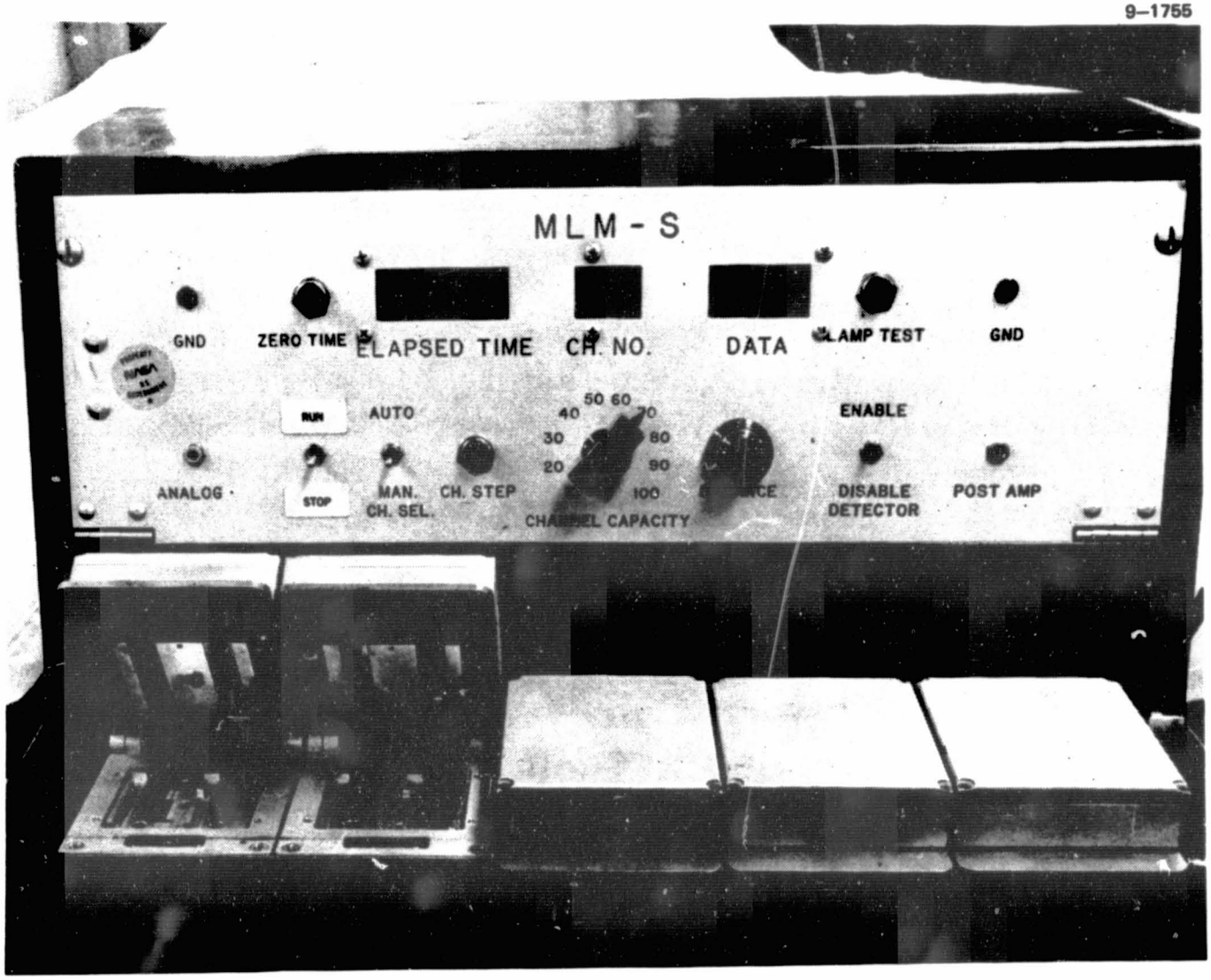

FIGURE 2–5
MLM-S

of microbes and have each identified microbe tested for sensitivity against four antibiotics. To test each microbe against 15 antibiotics as may be done in the clinical laboratory would require a Card that was too large to be efficiently used. Cassettes with freeze dried media were tested for long term storage and most were found to retain effectiveness after four months. We fabricated devices to semiautomatically load the Card with the sample. We designed equipment to semiautomatically load a Card with selective and antibiotic media. This was seen as necessary for future developments. It had become clear that manually pipetting a different media into each of 60 small, closely packed holes in each of thousands of Cards was going to be impossible without inducing errors into the Card.

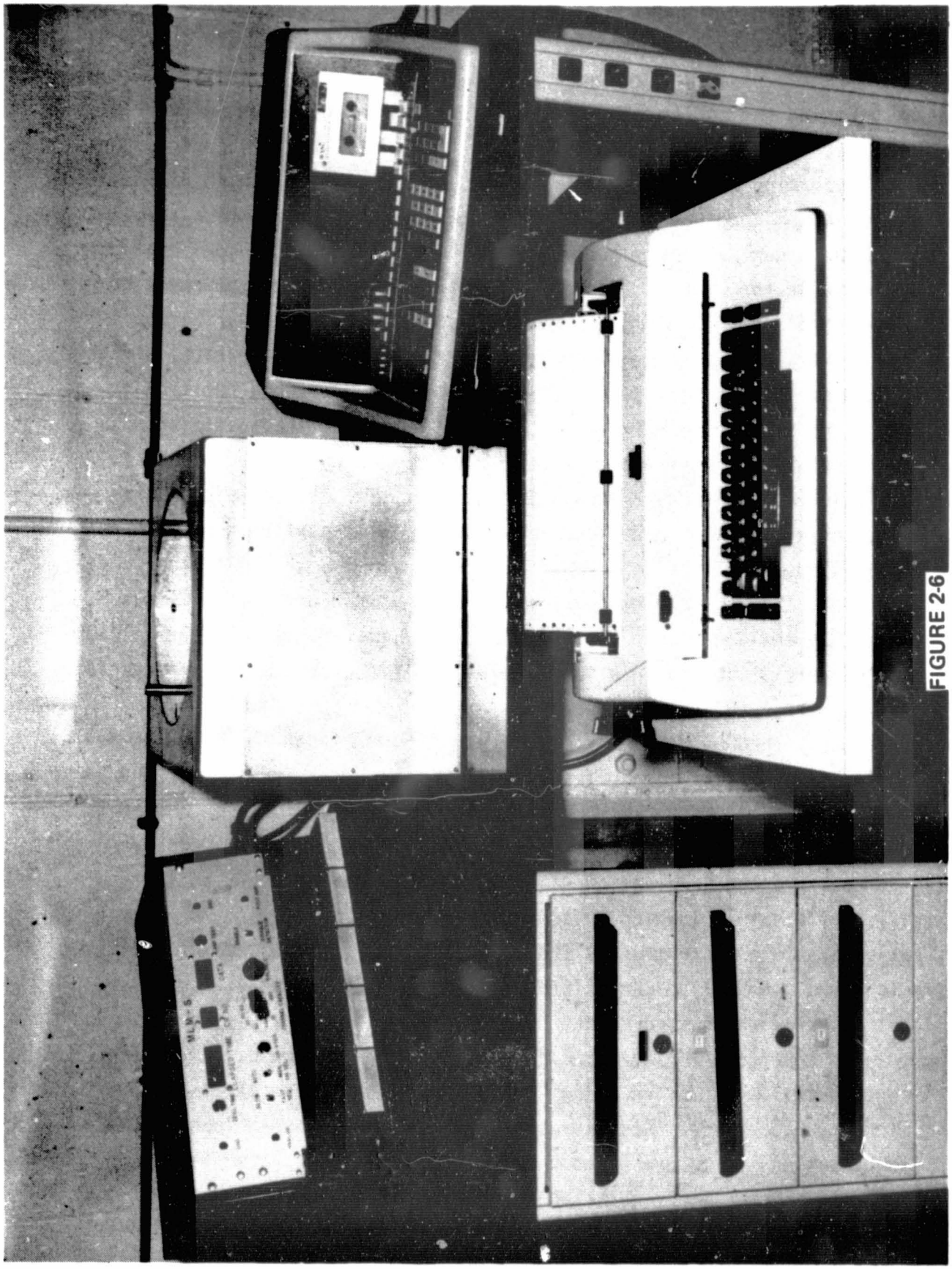

FIGURE 2-6
MLM-EA

Another digression is necessary at this point because events outside the developmental process began to influence later development - especially the media development. During the course of Exhibit B activity, patents were awarded to NASA on the basic MLM technology for automatically identifying pathogenic organisms and for performing antibiotic sensitivity tests on those organisms identified. Also, President Nixon announced his signing of an Executive Order which said in effect that those organizations who, in the course of government funded contracts, patent inventions in the name of the government may petition the government to waive its own rights. It says further that after review of the organization's petition for waivers, the government may grant the petitioner the exclusive rights to commercially exploit those patents. MDAC-St. Louis made such a petition to NASA for the right to commercially exploit the MLM patents. That petition was granted. As a result, an agreement between NASA and MDAC was signed. This agreement stipulated that further media development was in the commercial interests of MDAC; therefore, such further development would be performed by MDAC out of its own funds, but that the NASA would be supplied with such media at no cost to the government. The instrument development for a low-volume, space flight version of the MLM could continue to be funded by the government. Of course, the high-volume, commercial version was to be funded at MDAC expense. The commercial device is called the AutoMicrobic System. A wholly owned subsidiary, Vitek Systems, Inc. was formed by McDonnell Douglas Corporation to further develop and market the AMS to hospital laboratories.

During the contract period represented by Exhibit C, Cards were fabricated by machining to evaluate the Card design that was made during Exhibit B activity. A flight version of a sample loading system was designed for Card use. The present MLM was designed. This instrument is shown in Figure 2-7. It is capable of incubating and monitoring five Cards. This integrated MLM has a self contained sample loading system which is designed to operate in a null gravity environment. A microprocessor controls, monitors and performs calculations on data from the Cards. Preprogrammed information allows automatic determination of positive and negative microorganism tests. Results can be requested through an integral keyboard and information can be presented on its alphanumeric display. Hard copy final results can be printed by a peripheral teleprinter after completion of the 13 hours required to complete tests. Time history profiles can be plotted from

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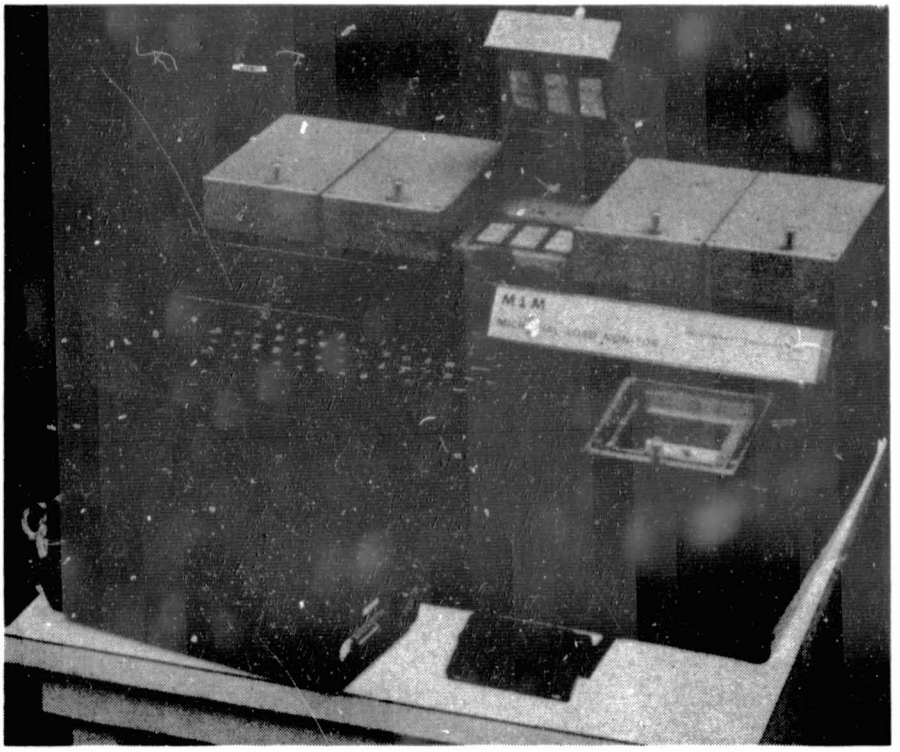

FIGURE 2–7
MLM INSTRUMENT

the integral magnetic tape. The cabinet and one reading head was fabricated and installed.

During the time interval of Exhibit C the commercial group (AutoMicrobic System) settled the enumeration problem. The classical means for counting total microorganisms in food and water samples is the method called the Most Probable Number. This method calls for a very large series of serial dilutions through to the dilution at which no organisms grow. From this data, in which dilutions there was growth and in which dilutions there was no growth, statistically derived tables indicate the Most Probable Number of organisms that were in the original sample. Where there is no apparent growth in 24 hours of incubation, there were so few organisms in the previous concentration that its sampling resulted in no organisms being seeded in the next dilution. This old technology was applied to the clinical question: "Are there more than 100,000 microbes per ml in the urine?" It is only in a urine sample that the physician has any interest in

enumeration. The AMS group showed that a degraded version of the Most Probable Number technique could be used to answer that clinical question. They made Cards that had five wells containing media which would support growth of all kinds of organisms. A diluted urine sample was drawn into those wells. By dilution the probability of a viable inoculum of microbes getting into and having detectable growth in each of the wells is very small. Empirically, it was determined that, if growth is detected in all five wells, the original sample had more than 100,000 microbes per ml.

One additional feature was offered by the AMS group. If the clinical sample contains a microorganism for which the Card has no selective media, the instrument would falsely indicate that no microbes were present in the sample. To avoid that kind of error, the present Card has one well which will support the growth of other microbes. That well is called the positive control.

At the completion of Exhibit C, all design activity on the MLM was completed. The remainder of the contracted period was spent in fabrication of components as funding became available. During Exhibit D injection molds for the Card and sample loading devices were fabricated and production runs were made for both kinds of devices. The keyboard and display were integrated into the instrument and operational software was developed. During the Exhibit E period, the sample loading system was fabricated and integrated into the instrument. Two additional reading heads were added.

During the final contract period, Exhibit F, the final two heads of the present instrument were fabricated and integrated into the MLM. The Card loading device was fabricated. The Card taping device was fabricated. The processing of the deliverable Cards was accomplished. The Cards were loaded with media prepared by Vitek but the freeze drying was done at MDAC in a small freeze dryer. These Cards did not maintain their shelf life because the media in them rehydrated. A second production run of Cards was performed at Vitek, again using media prepared by Vitek but also using their production freeze dryer. The second run of Cards was sealed individually in aluminum pouches as was the first run of Cards. However, to further prevent moisture from getting into the media, these bagged Cards were placed individually in a second plastic bag containing drying agent and sealed. These difficulties dramatically demonstrated that solving the

research problems was only one part of the total problem. The other is the reliable manufacturing of the Cards. Also accomplished during the contract period was the comparative tests between the MLM and AMS performance. They are equivalent. The validity of the MLM readings, therefore, rests on thousands of challenges of the AMS by double-blind studies performed by leading independent clinical laboratories.

During the course of this 13 year research and development period, literally hundreds of media have been formulated and tested to find 13 media that have withstood the challenges. This is a terribly arduous process and will be addressed here. The media testing at all stages started with test tube tests to determine if the media would permit one and only one kind of microorganism to grow. The first microbes used to challenge the media were commercially available laboratory organisms. There are thousands of those available. If the media stood up under those tests, wild strains from infected patients were tried - some of the media failed. If the media were still functioning properly, they were tested for freeze drying compatibility - many could not tolerate that treatment although that proved to be the best technique to obtain long term (six months) shelf life. If the media were still good they were tried in the then current MLM model. With many media a change was visible to the eye of the microbiologist but not to the instrument. New chemical gain indicators would be tried to make the change visible to the instrument. This, too, was not always successful. Even after passing all those hurdles, some media had to be changed again after the results of the Vitek, large-scale clinical trials were completed. This trial and error method has led to highly selective and sensitive media, but even with all these advances some organisms cannot be positively identified. There are still some microorganisms, such as beta hemolytic streptococcus Group A, which are very important clinically, but for which we have not been able to develop a media which passed all tests. But Vitek is continuing the process, and the instrument is very useful in its present status and will prove its value in the Shuttle era.

3.0 MATERIALS AND METHODS

3.1 BIOLOGICAL

Major headings described in this section are as follows: (1) Selective Media Development; (2) Media Forms and Preparation Methods; (3) Enumeration Studies; (4) Antimicrobial Susceptibility Tests; (5) MLM Card Configurations; (6) Storage and Return Capability; (7) Clinical and Seeded Samples Protocols; and (8) Quality Control and Shelf-Life Studies. Detailed data are provided below for each of the preceding categories.

3.1.1 Selective Media Development - Several hundred culture media formulations have been developed and tested for their selective ability on microorganisms groups or species in mixed populations. Selection was on a growth basis or growth plus a physiological event such as urease production, coagulase activity or pH change in the presence of inhibitors. In some cases, previously unknown carbohydrate sources were utilized. Up to date formula for each selective medium follows below. These formulae represent the most successful of the several hundred tested over the last eight years. Many fomulations successfully tested in their liquid form were rejected for use when it was discovered that the freeze-drying process altered the efficiency of the medium.

Coliform Medium (per 100 ml)

Gelysate	1.0 g	pH 7.5
Lactose	1.0 g	
Sodium Desoxycholate	1.0 g	
Brilliant Green	1.33 mg	
Distilled Water	100 ml	

Principal: Sodium desoxycholate, Brilliant green and Lactose select for the coliform organisms. The desoxycholate precipitates when acid production is significantly contributing to optical detection.

E. coli Medium (per 100 ml)

p-coumaric acid	0.1 g	pH 7.5
3,4-dihydroxybenzoic acid	0.1 g	
Gelysate	0.5 g	
Lactose	0.5 g	

E. coli Medium (per 100 ml) (Continued)

L-arabinose	0.5 g
Bile Salts	0.3 g
Yeast Extract	0.1 g
Aniline Blue	2.5 ml
Saponin	0.1 gm

Principal: The broth is cream to light blue in color. When positive, the broth changes to a milky blue due to conversion of arabinose and lactose to acid, changing the color of the indicator and precipitating the bile salts.

Pseudomonas aeruginosa Medium (per 100 ml)

Tryptic Soy Broth	3.0 g	pH 6.8
Sodium Nitrate	0.5 g	
Cetyltrimethylammonium bromide	0.03 g	

Principal: A positive reaction is indicated by turbidity with or without pigment; it indicates resistance to celyltrimethylammonuim bromide (cetrimide).

Proteus Medium (per 100 ml)

Proteose Peptone #3	0.1 g	pH 6.8
Urea	2.0 g	
Monobasic Potassium phosphate	0.08 g	
Dibasic Potassium phosphate	0.12 g	
Colistin	500 mcg	
Erythromycin	500 mcg	
Sodium Thioglycollate	0.2 g	
Brom Thymol Blue	0.01 g	

Principal: Yellowish green. A deep blue color indicates a positive reaction, i.e., urease activity in the presence of inhibitors for organisms other than Proteus.

Citrobacter freundii Medium (per 100 ml)

Trypticase	0.5 g	pH 7.5
Bile Salts	1.0 g	
Palatinose	1.0 g	
Rhamnose	1.0 g	

Citrobacter freundii Medium (per 100 ml) (Continued)

Brilliant Green	53.2 mg
DP 300	2.5 ml of 1% stock

Principal: A positive reaction is indicated by a milky turbidity resulting from utilization of rhamnose and palatinose. Acid production precipitates the bile salts in the broth.

Serratia Medium (per 100 ml)

p-Coumaric Acid	0.3 g	pH 8.0
Gelysate	0.2 g	
Thiotone	0.1 g	
Bile Salts	1.0 g	
Plant Indican	0.1 g	
DP 300	2 ml of 1% stock	

Principal: When positive, a smoky-black color develops due to the alteration of plant glycosides in the medium.

Group D Enterococcus Medium (per 100 ml)

Esculin Hydrate	0.1 g	pH 6.6
Trypticase	1.5 g	
Phytone	0.5 g	
Ferric Ammonium Citrate	0.05 g	
Neomycin Sulfate	1000 mcg	
Ampotericin B	500 mcg	
Potassium Tellurite	1 ml 1% stock	
Sodium Thioglycollate	0.2 g	

Principal: A positive reaction is indicated by the development of a black precipitate, formed when esculin is hydrolyzed in the presence of iron compounds.

Staphylococcus Aureus Medium (per 100 ml)

DNA-Methyl Green	50 mg	pH 7.7
Uracil	0.05 g	
Sodium Chloride	8 g	
Supplement B	2 ml	
Ampotericin B	500 µg	
d-mannitol	1 g	

Staphylococcus Aureus Medium (per 100 ml) (Continued)

Proteose Peptone #3	0.5 g
Yeast Extract	0.5 g
Potassium Tellurite	0.2 ml 1% stock
Supplement XV	0.025 g
Rabbit Serum	4 ml
Rabbit Plasma	4 ml
Vitamin B12	0.05 g

Principal: The broth is light pink to reddish purple. When positive it changes to a deep bluish purple and a coagulum is formed. The broth indicates tolerance to NaCl, DNA-ase activity, and plasma coagulation.

Yeast Medium (per 100 ml)

Phytone	1 g	ph 6.9
Dextrose	1 g	
Chloramphenicol	0.08 g	
Potassium Tellurite	0.3 ml of 1% stock	
Aniline Blue	2 ml	
Sodium Thioglycollate	0.2 g	

Principal: When positive, the broth changes to an intense blue occurring when dextrose is fermented, resulting in acid production and pH change. Inhibitors prevent the positive reaction from occurring with bacteria.

Positive Control/Enumeration Medium (per 100 ml)

Gelysate	0.3 g	pH 7.0
Proteose Peptone #3	0.3 g	
Lactalysate	0.3 g	
Yeast Extract	0.3 g	
Dextrose	0.3 g	
d-mannitol	0.3 g	
Supplement B	0.5 ml	
Aniline Blue	1.0 nl	
Sodium Thioglycollate	0.1 g	

Principal: When reconstituted, the broth is cream to very light blue. When positive the color changes to bright blue. This is a general purpose broth containing multiple peptones, vitamins, and growth factors X and V. A color change results when acid is produced.

Streptococcus Medium #1 (per 100 ml)

Phytone	0.5 gm	pH 7.3
Salicin	0.1 gm	
Trypticase	1.5 gm	
NaCl	0.5 gm	
Washed Red Blood Cells	1 ml	
Neomycin Sulfate	0.025 mg	
Potassium Tellurite	0.5 ml of 1% solution	

Principal: The antibiotic and tellurite prevent the rapid growth of organisms other than streptococci. The lysis of the blood provides an indicator system for MLM detection. This medium cannot be freeze dried.

Streptococcus Medium #2

Human plasma, oxalated (fasting)	60 ml	pH 7.6
Buffered Saline solution pH 6.8	20 ml	
Thromboplastin (Fibroplastin)	20 ml	
1% stock solution Potassium Tellurite	.1 ml	

Principal: The fibrinolytic action of the group A beta hemolytic streptococci is detected by the MLM. This medium can be freeze dried.

Streptococcus Medium #3 (per 100 ml)

Proteose peptone #3	0.5 g	pH 7.6
Thiotone	0.5 g	
Trehalose	1 g	
Supplement B	1 ml	
Thiamine HCl	0.4 g	
Lithium Chloride	0.3 g	
Thallous Acetate	0.01 g	
Saponin	0.05 g	
Supplement XV	0.025 g	
Rabbit Plasma	10 ml	
DNA-Methyl Green	25 mg	
Vitamin B12	0.4 g	
Potassium Cyanide	0.4 ml of 1% stock	
Kanamycin Sulfate	1000 Mcg	

Principal: The potassium cyanide and kanamycin prevent the rapid growth of organisms other than streptococci. Group A Beta streptococci are indicated by growth in the medium and lack of growth in this medium containing bacitracin. This medium has limited freeze-dried performance.

Klebsiella-Enterobacter Medium (per 100 ml)

3 (α acetonylbenzyl)-4-Hydroxycoumarin	0.2 g	pH 8.0
Gelysate	0.5 g	
Cellobiose	1.0 g	
Inositol	1.0 g	
Sodium Desoxycholate	1.0 g	
Ricinoleic Acid	1.0 g	
d-biotin	0.05 g	
Yeast Extract	0.05 g	
Brilliant Green	0.013 g	
K/E Indicator*	0.003 g	

*(2, 4-dinitrophenyl hydrazone of α-Ketoglutaric acid)

Principal: When positive the broth appears milky green after utilization of cellobiose and inositol in the presence of inhibitors.

Salmonella Medium (per 100 ml)

Sodium acid selenite	0.5 gm	pH 6.7
L-lysine mono HCl	1.0 gm	
Ammonium Chloride	0.3 gm	
Yeast Extract	0.1 gm	
KH_2PO_4	0.18 gm	
Phenol Red	0.003 gm	

Do not heat

Principal: The selenite present in the broth selects for Salmonella and a few other gram negative organisms. However, precipitation of the selenite is caused by Salmonella species and this is detected by the MLM.

Hemophilus Medium (per 100 ml)

2 ml Tween 80	pH 7.6
Menadione	0.4 gm
Human Lyophilized plasma	3 gm
H_2HPO_4	.1 gm
Supplement XV Factor (Difco)	1.5 ml
Filter excess Menadione	

Principal: Factors present in the Difco product XV react with menadione to produce a spectrum of colored compounds depending upon the presence of other compounds containing nitrogen and carboxyl groups. The Hemophilus species of organisms have the metabolic power to rapidly attack these compounds to produce a wine color which is detectable by the MLM. Menadione and its degradation products are toxic to other microorganisms. This medium can be freeze dried in two parts and mixed dry to produce a freeze dried medium.

Streptococcus Pneumoniae Medium

Brain Heart Infusion broth	0.3 gm
Inulin	1.0
Bovine albumin	1.5 gm
Phenethyl alcohol	.05 gm
Carboxyhemoglobin	20 ml stock solution*
Neomycin Sulfate	25 micrograms
Distilled water	q.s. to 100 ml

final pH adjusted to pH 7.8

*Stock carboxyhemoglobin prepared by placing 6 ml washed packed human red cells in 100 ml distilled water, and bubbling CO through the solution for 10 minutes.

Principal: The phenethyl alcohol and antibiotic select for gram positive organisms. The carbohydrate source and protein source encourage Streptococcus pneumoniae to grow faster than the competitors. The carboxyhemoglobin provides an indicator system detectable by the MLM which is chemically stable and is toxic to some competing organisms. This medium can be freeze dried.

Neisseria Meningitidis Medium (per 100 ml)

Trypticase	0.75 gm	pH 7.3
Thiotone	0.75 gm	
K_2HPO_4	0.2 gm	
KH_2PO_4	0.05 gm	
NaCl	0.5 gm	
Dextrose	0.5 gm	
Hemoglobin	0.25 gm	
Supplement B (Difco)	2 ml	
Nystatin	10,000 Units	
Vancomycin	3,000 mcg	
Colistin	750 mcg	
Trimethylprim Lactate	5 mg	

Principal: Antibiotic combinations select for Neisseria meningitidis and some strains of Proteus. Proteus is eliminated by the addition of trimethylprim lactate. This medium can be freeze dried.

Acinetobacter/Herellea Medium (per 100 ml)

2-desoxy-d-glucose	0.2 gm	pH 9.1
Myosate	0.1 gm	
Yeast extract	0.08 gm	
Tris buffer	0.6 gm	
2-aminothiazoline	0.004 gm	
Nitrofurantoin	20 mcg/ml	
Nystatin	5,000 Units	

Principal: This medium provides a harsh environment with a high pH and a minimum of nutrients to take advantage of the ability of the Acinetobacter/Herellea to grow in such environments. This medium can be freeze dried.

Aspergillus Medium (per 100 ml)

Sucrose	3.0 gm
$NaNO_3$	0.3 gm
$MgSO_4$	0.05 gm
KCl	0.05 gm
NA_2HPO_4	0.12 gm
KH_2PO_4	0.005 gm

Aspergillus Medium (per 100 ml) (Continued)

Chloramphenicol	0.006 gm
Phenol red	0.0025 gm pH 7.3
Indicator	1 ml stock*
Colistin	0.25 mg
Nalidixic Acid	0.25 mg

*0.1 gm aniline blue
0.1 gm sodium Thioglycollate
10 ml dist H_2O pH 9.0

Principal: A nitrogen source containing only ammonium and nitrate salts combined with a broad spectrum antibiotic select for Aspergillus species. The reduced aniline blue is the indicator system. This medium can be freeze dried.

3.1.2 Media Forms and Preparation Methods - Media was prepared in various forms in order to evaluate shelf life, performance, and ease in future high volume Card preparation.

The formulations were prepared in the following forms: Freeze-dried preparation; pellets; air-dried; saturated dried porous disc; and plasticized wafer.

Freeze-dried preparation - Media were prepared and loaded into Card wells using a microliter pipettor. Cards were placed in an ultra-low (-70°C) freezer for 4-6 hours, then placed into a Virtus freeze drier for 24 hour freeze-drying cycle.

Pellets - Media constituents were prepared with minimal moisture and formed into miniature pellets which were placed into the Card wells.

Air dried preparation - Media were mixed as in freeze-dried procedure but filled Cards were allowed to air dry.

Saturated porous disc - Media were prepared as in air dried method. Paper filter discs were saturated with the various media and air dried before placing into the Card wells.

Plasticized Media Wafer - Media constituents were added to a glycerin and gelatin solution in water. The mixture was poured onto flat trays and allowed to

harden. When dried, the sheets were punched and small wafer discs were loaded into Card wells. Detailed description is shown on Figure 3-1.

3.1.3 Enumeration Studies - Several years of effort were devoted to development of a reliable method for enumerating the population of raw clinical specimens - most notably urine samples. Significant bacteriuria is $\geq 10^5$ colony forming units (CFU)/ml.

Two approaches of enumeration for MLM were studied. The first method utilizing filtration discs and serial dilution was not chosen as the final method for MLM incorporation, but will be discussed. The second approach, a modification of The Most Probable Number Theory is discussed in detail and is the current method for enumeration.

Disc Filtration Method

Principal: The disc filtration method of enumeration assumed that the bacteria population of a sample in liquid would be progressively reduced as it passed through a series of filtrations and that rate of population reduction could be calculated for a given sample.

Serial dilution of the liquid sample was accomplished in the filter cassette as shown in Figure 3-2. The sample was injected into the cassette through the sample port (septum). The septum was pierced with a needle attached to syringe containing the liquid sample. The cassette was evacuated and the repressurization cycle allowed flow of the liquid through the serial wells. The filter wells contained the filter material. Various filter materials and thicknesses were evaluated and included Whatman numbers 1, 3, 42 and 541. Also tested were Reene Angel No. 802, 934AH (glass filter), acetate, and asbestos fibers for effectiveness in serial log reduction of bacteria.

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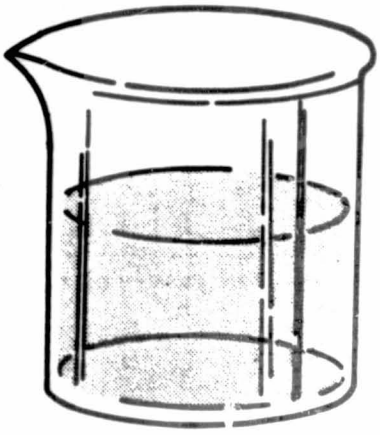
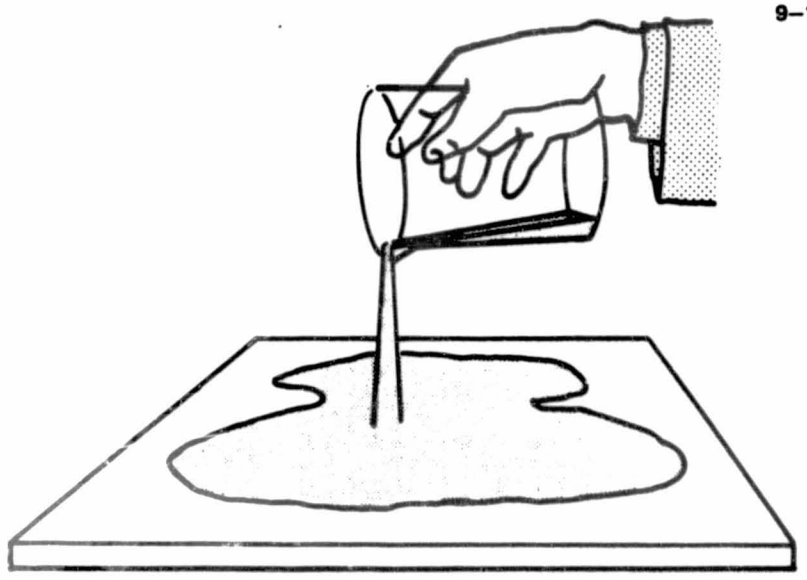
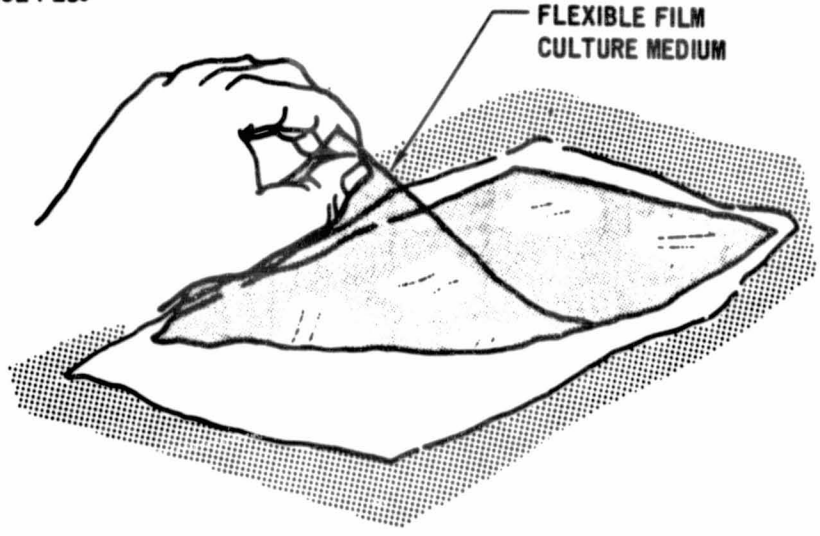


1. PER 100 M L H_2O:
   3.0 GRAMS MICROBIOLOGICAL GRADE GELATIN 0.8 ML CHEMICALLY PURE GLYCERIN WARMED TO 40°C UNTIL GELATIN DISSOLVED. CULTURE MEDIA ADDED
   OR
   2.0 GRAMS 99% HYDROLYSED POLYVINYL ALCOHOL PLUS CULTURE MEDIA



2. WHILE STILL WARM, LIQUID IS POURED ONTO FLAT PLEXI-GLAS SURFACE AT THE RATIO OF 200 ML OF WARM SOLUTION FOR EACH SQUARE FOOT OF SURFACE.



3. AFTER DRYING, FILM IS STRIPPED OFF PLASTIC SURFACE AND CUT TO SIZE. STERILIZATION BY ETHYLENE OXIDE IF NEEDED.

FIGURE 3–1
STEPS IN PROCESS FOR PREPARING PLASTIC MLM CULTURE MEDIA FOR PUNCHING INTO CASSETTES

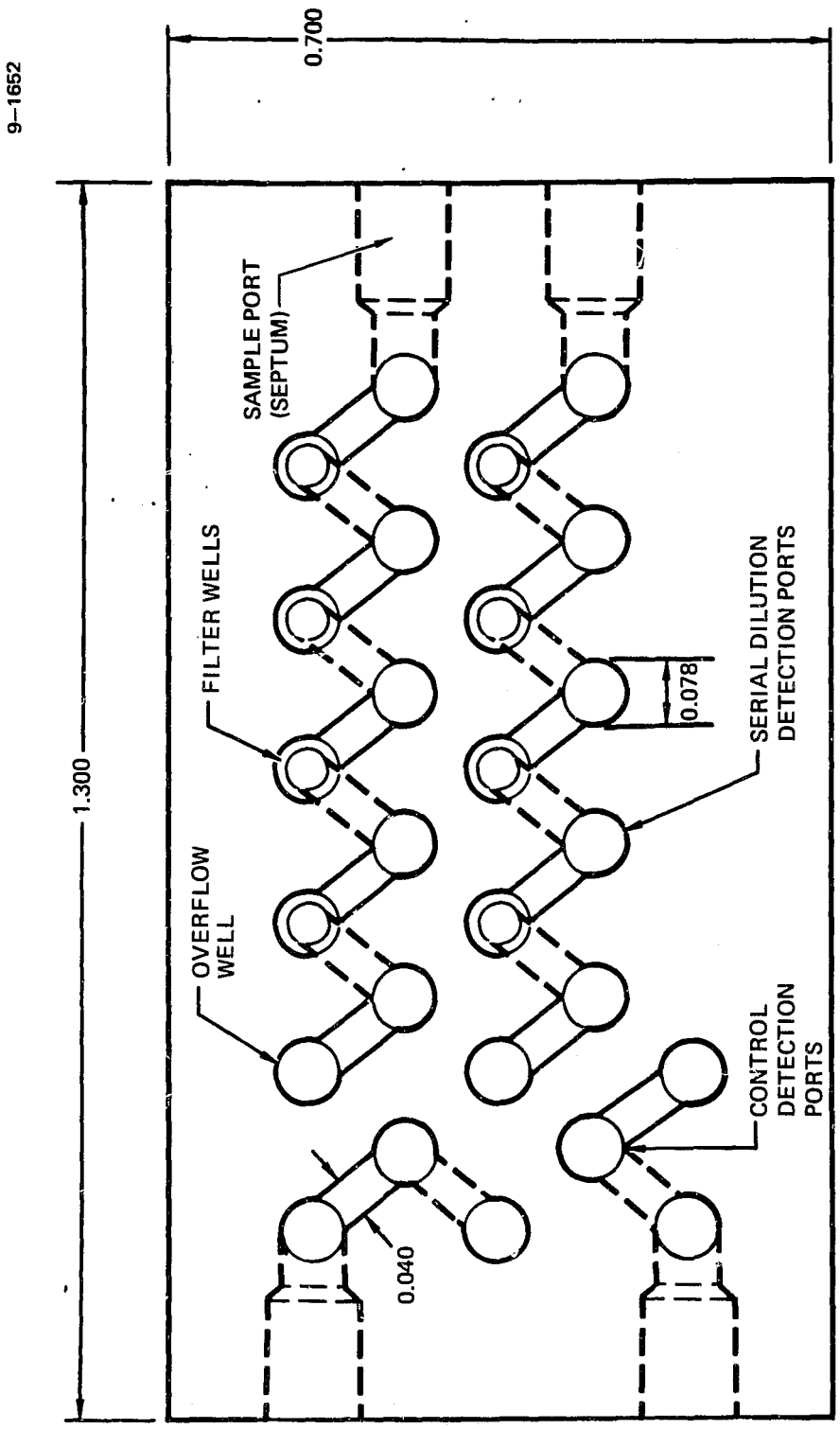


FIGURE 3–2
DUAL FILTER CASSETTE

Current MLM Enumeration - The current MLM enumeration scheme is based on the Most Probable Number (MPN) theory. MPN estimates are based on the assumption that bacteria are evenly distributed in liquid media such that repeated samples of the same size from one source will contain the same number of organisms on the average. A common method of utilizing the MPN method consists of inoculating three sets of five tubes containing broth with either three different volumes or three different dilutions of sample. The tubes are then incubated 24 to 48 hours for observation of growth. Concentration of organsims in the original sample is based on the number of positive tubes. A complete description of the MPN procedure along with tables of precalculated MPNs based on the possible combinations of the positive and negative tubes is found in Reference 1. The MLM enumeration scheme is an MPN method in which a series of five wells are observed for growth, color change, or turbidity.

A mathematical model of the enumeration section of the MLM Clinical Card can be configured using Poisson distributions. An individual well of the MLM Card has a total volume of 32 μl.

The number of organisms in a well of volume V filled with diluted sample X/d is a Poisson random variable with mean AX, where AX = (V/d) X; such that X is the concentration of the sample in organisms/ml, and d is the factor by which the original sample is diluted in the enumeration section of the Card (i.e., the concentration loaded into the enumeration SRCLD). The probability distribution of y, the number of organisms per well for a known concentration X, is:

$$P\,[y = K/X] = \frac{e^{-AX}(AX)^k}{k!},\quad K = 0, 1, 2 \ldots$$

The probability of zero bacteria in an enumeration well is:

$$0.11 \text{ for } X = 7X10^4 \text{ organisms/ml}$$

$$0.04 \text{ for } X = 1x10^5 \text{ organisms/ml}$$

The probability of positive bacteria in an enumeration well is:

$$0.89 \text{ for } X = 7X10^4 \text{ organisms/ml}$$

$$0.96 \text{ for } X = 1X10^5 \text{ organisms/ml}$$

The enumeration medium employed in the MLM Card is a general purpose enrichment formulation designed to detect fastidious species as well as those species commonly encountered in urine samples. For the MLM five positive wells indicates $\geq 10^5$ organisms/ml with a high degree of assurance.

3.1.4 Antimicrobial Susceptibility Tests - Efforts to expand the concept of selective growth and identity of microorganisms from raw specimens to include simultaneous antimicrobial susceptibility testing required a major developmental effort beginning in early 1972 and extended to the conclusion of this contract.

Approach to MLM Susceptibility Testing - MLM adaption of the antimicrobial susceptibility test program was divided into four areas of study and testing which was regarded as four logical steps required to accomplish the task.

Step 1: Develop the rationale for choice of antibiotics to be tested with each organism of medical importance as identified by NASA.

Step 2: Determine the optimum antimicrobial concentration in each selective medium which best correlates with standard results.

Step 3: Incorporate any formulation changes required for antimicrobial-media compatibility.

Step 4: Performance tests with clinical samples in MLM media versus parallel standard culture and susceptibility test methods.

Local hospital susceptibility test results were monitored over a several year period in order to incorporate the drugs currently in use noting those that were most effective or giving a high percentage of "susceptible" results. As the contract progressed, some antimicrobials were replaced with more currently used compounds. The current media-drug combinations are listed in Table 3-1.

TABLE 3–1

CURRENT MEDIA – ANTIMICROBIAL COMBINATIONS FOR MLM CARD

9–1648

E .coli	P. aeruginosa	Acinetobacter-Herellea
Nitrofurantoin*	Amikacin	Kanamycin
Trimethoprimsulfamethoxazole*	Tobramycin	Gentamicin
Nalidixic Acid*	Gentamicin	Nalidixic Acid*
Ampicillin	Carbenicillin	Tetracycline
Kleibsiella-Enterobacter	Serratia Sp.	Beta Streptococcus
Gentamicin	Gentamicin	Bacitracin**
Trimethoprimsulfamethoxazole*	Trimethoprimsulfamethoxazole*	Ampicillin
Nalidixic Acid	Nalidixic Acid*	Penicillin
Tetracycline	Kanamycin	Erythromycin
Citrobacter freundii	Group D Enterococcus	
Nitrofurantoin*	Nitrofurantoin*	
Trimethoprimsulfamethoxazole*	Erythromycin	
Nalidixic Acid*	Tetracycline	
Tetracycline	Ampicillin	
Proteus Sp.	S. aureus	
Kanamycin	Cephalothin	
Trimethoprimsulfamethoxazole*	Erythromycin	
Nalidixic Acid*	Tetracycline	
Ampicillin	Clindamycin	

*For urinary tract infections only
**For identity of Group A Beta streptococci

Optimum antimicrobial concentrations for each medium were chosen by performing serial dilutions of the drugs in media. The media-drug combinations were prepared and incorporated into the Cards by one of the methods described in Section 3.1.2. Organisms were prepared as a 10^6/ml slurry in 0.5% NaCl and inoculated into the Cards. The Cards were placed in the MLM and growth was noted over a 13 hour incubation period. "Sensitivity" was measured as no growth in the antibiotic-media well and growth in its corresponding control well (well containing selective medium and no antimicrobial). "Resistance" was measured as growth in both wells. Results were correlated to standard susceptibility methods. The standard test used was the Kirby-Bauer disc diffusion method as outlined in the NCCLS Manual (2). Many thousands of such tests were required to obtain an optimum antimicrobial concentration for each drug in each selective medium. From the results of these tests a single concentration for each drug was established in its respective selective medium. These media drug concentrations are listed in Table 3-2.

TABLE 3–2
MEDIA-DRUG CONCENTRATIONS FOR MLM SELECTED MEDIA

9–1647

Drug & Concentration (mcg/mL)	E. coli	Kleb-ENT	C. Freundi	Proteus	P. aeruginosa	Serratia	Group D. ENT	S. aureus	Acinetobacter Herellea	Beta Strep
Nitrofurantoin	30	X	30	X	X	X	40	X	X	X
Trimeth-Sulfa	10	2.5	20	30	X	30	X	X	X	X
Nalidixic Acid	20	1.5	20	50	X	30	X	X	30	X
Tetracycline	X	7.5	20	X	X	X	15	5	20	X
Ampicillin	25	X	X	50	X	X	10	X	X	10
Gentamicin	X	5	X	X	20	20	X	X	10	X
Kanamycin	X	X	X	20	X	30	X	X	10	X
Amikacin	X	X	X	X	6	X	X	X	X	X
Tobramycin	X	X	X	X	4	X	X	X	X	X
Carbenicillin	X	X	X	X	40	X	X	X	X	X
Cephalothin	X	X	X	X	X	X	X	10	X	X
Erythromycin	X	X	X	X	X	X	2	5	X	2
Clindamycin	X	X	X	X	X	X	X	5	X	X
Penicillin	X	X	X	X	X	X	X	X	X	10*

*Units/mL

In cases of obvious drug-medium incompatibility, further work was required. The formulations were modified to retain their selectivity while at the same time provide a reliable "sensitivity" or "resistance" correlation to standard methods.

This was accomplished in some cases by eliminating some media constituents where these deletions did not significantly alter overall media selectivity. An example is the current Proteus broth formulation in which Mg^{++} was found to interfere with kanamycin and gentamicin performance. The removal of Mg^{++} from the formulation did not alter overall media selectivity characteristics.

The final media-drug combinations were prepared and tested with clinical isolates as seeded samples following the protocol given in Section 3.1.7. When sufficient data was accumulated to show reliability of the antimicrobials in their selective media, clinical samples including both pure and mixed samples were tested using the protocol as given in Section 3.1.7.

3.1.5 MLM Card Configuration - The current MLM Card evolved from a series of concepts, designs, and working models and covers a development and test period of several years. The various Card models are discussed in detail under Section 3.2, Hardware Development. Figure 3-3 is a photograph of the current MLM Clinical Card and Figure 3-4 shows the three-sectioned MLM Environmental Card. Media and drug combinations by well number are listed in Table 3-3 for the Clinical Card. The Clinical Card is divided into two individual sections each containing its respective septum. The main set of wells (numbers 1 - 55) contain the positive control well, selective media, and antimicrobial combinations. These wells are filled from a diluted row sample as outlined in Section 3.1.7. The remaining five wells contain the nonselective broth and are used for enumeration. A second dilution of sample (Section 3.1.7) is used to rehydrate the media in these wells.

The Environmental Card consists of three separate 20-well units that contain selective media but no antimicrobials. One to three samples may be used for each Environmental Card. Media location and well numbers are given in Table 3-4.

The media formulations used in the MLM Environmental Card are the same as those of the Clinical Card with the following deletions or additions:

9–1626

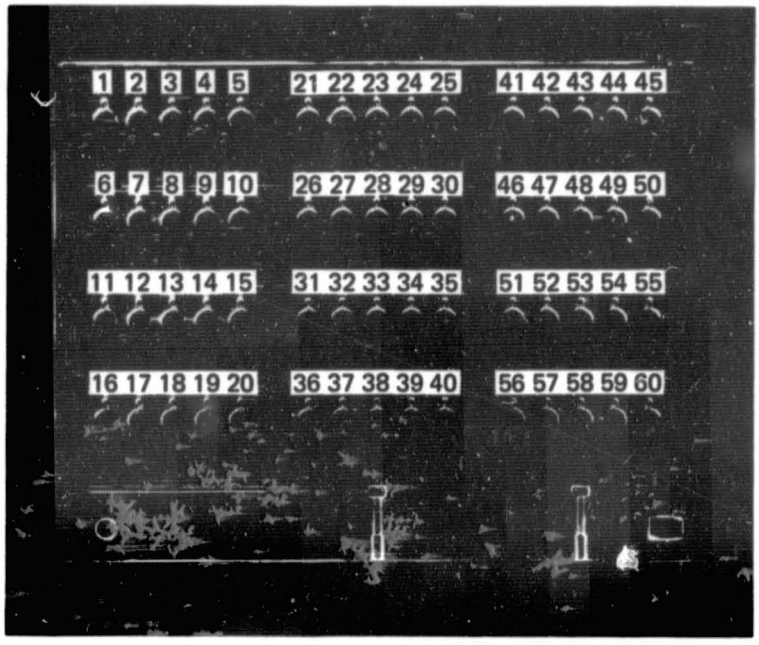


FIGURE 3–3
MOLDED CLINICAL CARD

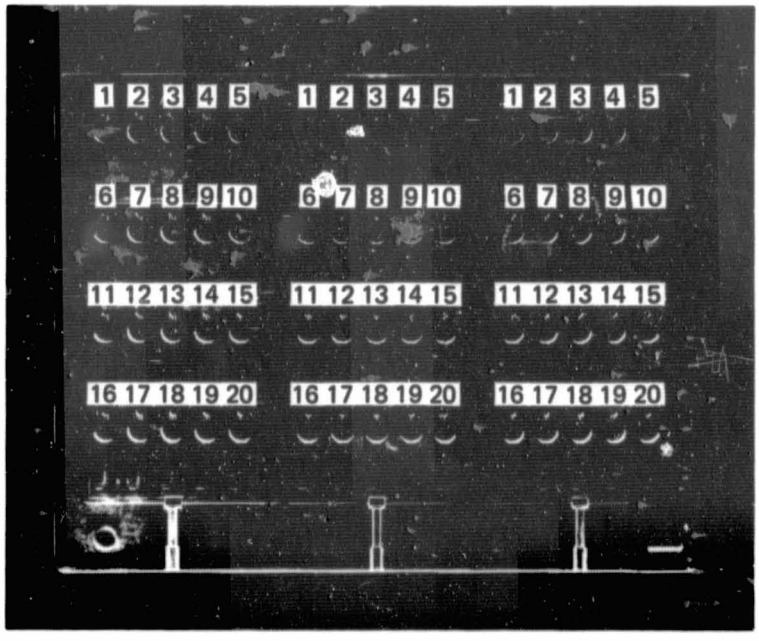


FIGURE 3–4
MOLDED ENVIRONMENTAL CARD

TABLE 3–3
MEDIA OR MEDIA DRUG COMBINATIONS BY WELL NUMBER MLM CLINICAL CARD

9 1645

Well No.	Identity	Well No.	Identity
1. E. coli	Control	31. GRP D Enterococcus	Control
2. E. coli	Fd	32. GRP D	Fd
3. E. coli	SXT	33. GRP D	E
4. E. coli	Na	34. GRP D	Te
5. E. coli	Amp	35. GRP D	Am
6. KLEB-ENT.	Control	36. S. aureus	Control
7. KLEB-ENT.	Gm	37. S. aureus	CF
8. KLEB-ENT.	SXT	38. S. aureus	E
9. KLEB-ENT.	Na	39. S. aureus	Te
10. KLEB-ENT.	Te	40. S. aureus	CC
11. C. freundi	Control	41. Acinetobacter/Herellea	Control
12. C. freundi	Fd	42. Acinetobacter	K
13. C. freundi	SXT	43. Acinetobacter	Gm
14. C. freundi	Na	44. Acinetobacter	Na
15. C. freundi	Te	45. Acinetobacter	Te
16. PROTEUS	Control	46.	Beta Strep Control
17. PROTEUS	K	47. GRP A	Beta
18. PROTEUS	SXT	48. Beta	Am
19. PROTEUS	Na	49. Beta	P
20. PROTEUS	Amp	50. Beta	E
21. P. aeruginosa	Control	51. YEAST	
22. P. aeruginosa	AN	52. –	
23. P. aeruginosa	NN	53. –	
24. P. aeruginosa	Gm	54. –	
25. P. aeruginosa	Cb	55. Positive Control	
26. Serratia	Control	56. Enumeration	
27. Serratia	Gm	57. Enumeration	
28. Serratia	SXT	58. Enumeration	
29. Serratia	Na	59. Enumeration	
30. Serratia	K	60. Enumeration	

a. No drugs are added; therefore, no antimicrobial susceptibility data is available.
b. Addition of fungi (aspergillus) medium applicable for environmental sampling.
c. No enumeration system; therefore, total numbers of microorganisms not determined.

3.1.6 Storage and Return Capability of Positive MLM Samples - The goal of these studies was the determination of the optimum treatment of the MLM detected microorganisms to afford maximum survival after storage and return to earth receiving stations. Thus, MLM data can be corroborated by standard testing of the microorganisms.

TABLE 3–4
MEDIA BY WELL NUMBER
MLM ENVIRONMENTAL
3-SPECIMEN CARD

9–1649

Well No.	Identity	Well No.	Identity	Well No.	Identity
1	E. coli	1	E. coli	1	E. coli
2	Kleb-Ent	2	Kleb-Ent	2	Kleb-Ent
3	C. freundii	3	C. freundii	3	C. freundii
4	Proteus	4	Proteus	4	Proteus
5	P. aeruginosa	5	P. aeruginosa	5	P. aeruginosa
6	Serratia	6	Serratia	6	Serratia
7	Grp D Entero	7	Grp D Entero	7	Grp D Entero
8	Acinetobacter-Herellea	8	Acinetobacter-Herellea	8	Acinetobacter-Herellea
9	S. aureus	9	S. aureus	9	S. aureus
10	Yeast	10	Yeast	10	Yeast
11	Beta Strep	11	Beta Strep	11	Beta Strep
12	Fungi (Aspergillus)	12	Fungi (Aspergillus)	12	Fungi (Aspergillus)
13	Positive control	13	Positive control	13	Positive control

Various freeze procedures were evaluated for their effectiveness in allowing viable organism recovery following storage and subsequent thawing.

A quick freeze procedure was tested through 130 days storage. In this test procedure, organisms were grown in their respective media and placed in a REVCO ultra-low temperature freezer maintained at -80°F.

A slow freezing process was evaluated by growing the test organisms in their selective media for 20 hours and then placing the sample in an Associated Testing Laboratories, Inc. oven (Model RK-1100). CO_2 was introduced into the oven in a manner which permitted temperature control. The temperature was lowered 1°C every minute. In each case, the samples were removed from the oven at -40°C and quickly transferred to the REVCO ultra-low temperature freezer for long term storage testing. Freezing profiles for the three slow-freeze tests and quick-freeze tests are shown in Figure 3-5.

Cyroprotective Agents - A thorough literature search indicated that there are several compounds which afford cryoprotective activity (3, 4). Three of these agents were chosen for storage and return capabilities: glycerol 15% W/V, polyvinyl-pyrrolidinone (mol. wt. 40,000) 15% W/V, and dimethysulfoxide 10% W/V. Three organisms were chosen to test with these cryoprotective agents; Staphylococcus aureus, Streptococcus pneumoniae and Neisseria meningitidis. S. aureus was chosen for this

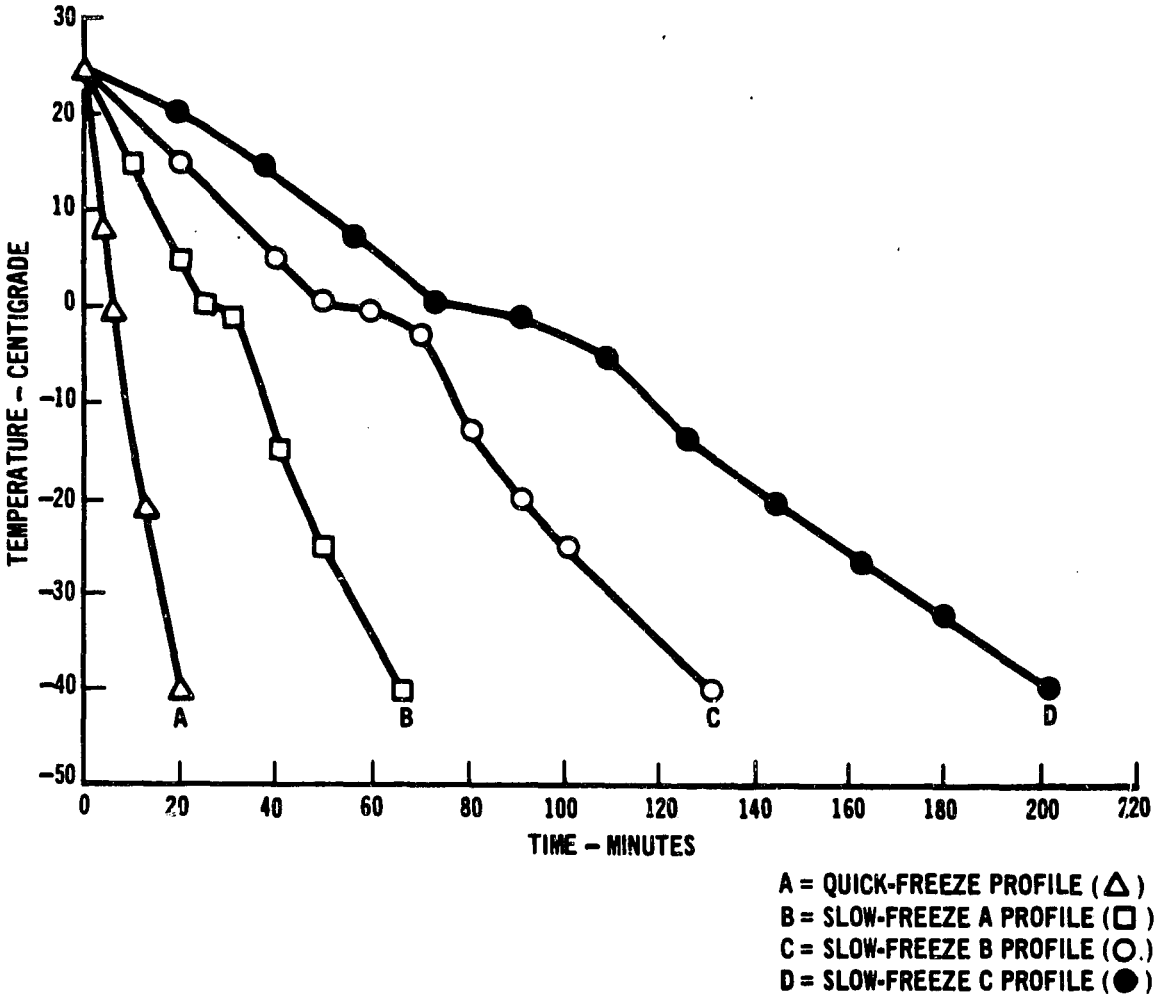


FIGURE 3-5
FREEZING PROFILES

study primarily because it was a hardy organism and would show whether any of the cryoprotective agents had adverse effects on the viability of hardy organisms. S. pneumoniae and N. meningitidis were chosen because of their poor performance in quick freeze tests (see Results, Section 4.1.5) and because they represented both gram positive and gram negative bacteria.

The precise method of testing consisted of growing the bacteria in their selective media at 37°C for 20 hours. The 20-hour cultures were then mixed with equal aliquots of each cryoprotective agent at a 2X concentration. This yielded the desired final concentration of each cryoprotective agent. The mixtures were then frozen rapidly in a REVCO ultra-low temperature freezer and stored at -80°F.

Freeze-Drying Techniques - In these tests, organisms grown in their selective media for 20 hours at 37°C were lyophilized in their respective media. Lyophilization was evaluated with and without addition of cryoprotective agents.

3.1.7 Protocols - Seeded and Clinical Samples - The protocols followed for MLM evaluation of seeded and clinical samples are given in Figures 3-6 through 3-8. The flow diagrams are self-explanatory. The standard microbiological methods used in these studies were the current state-of-the-art tests employed in clinical microbiological laboratories.

9–1646

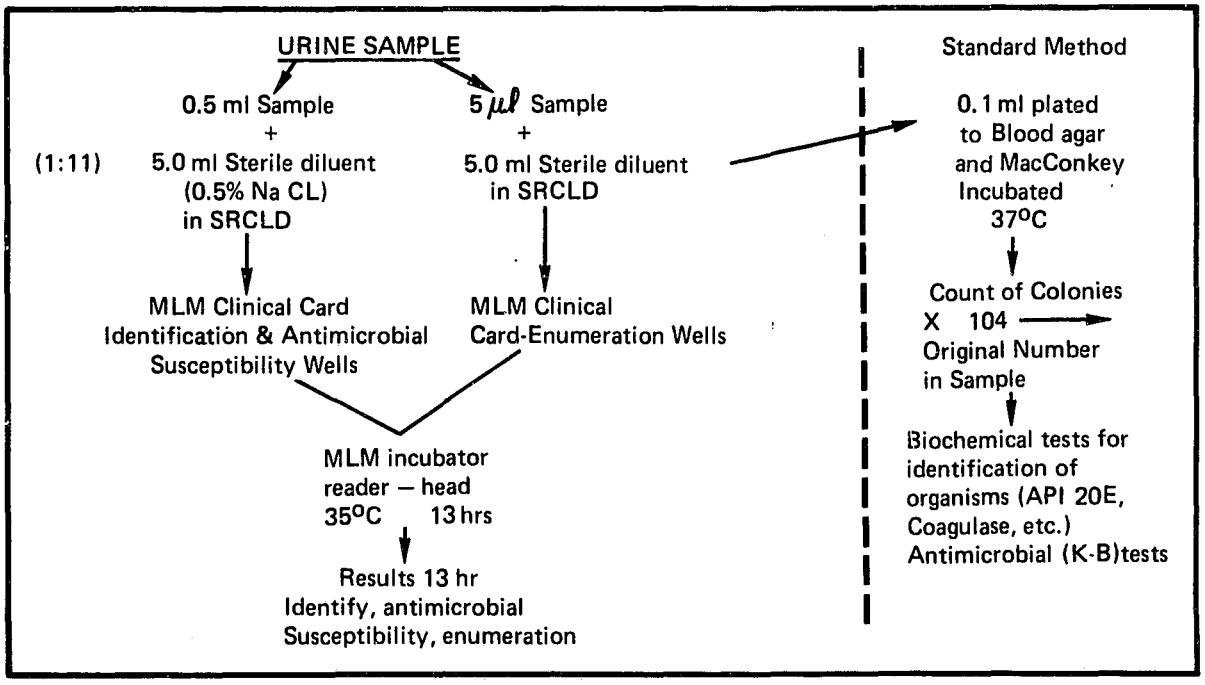


FIGURE 3–6
PROTOCOL FOR CLINICAL SAMPLES TESTS WITH URINE

Standard culture media, i.e., Blood agar, MacConkey, and chocolate agar were obtained from a commercial supplier (Remel; Lenexa, Kansas). Biochemical identification of microorganisms was done using the API 20 Enteric System (Analytab Products; Plainview, New York). When necessary, biochemical tests according to Ewing were performed for final identification (5); nonfermenters were identified by standard procedures (6). Staphylococci were tested for deoxyribonuclease activity, tolerance to 6.5% NaCl, and Coagulase activity. Streptococci were tested for ability to

9–1644

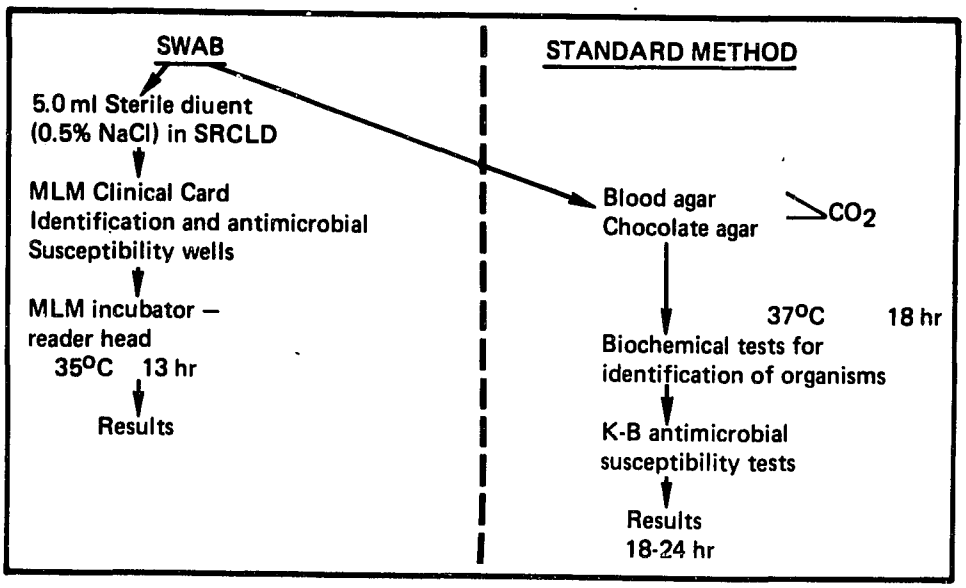


FIGURE 3–7
PROTOCOL FOR CLINICAL SAMPLES TESTS
THROAT OR SEPTUM SWAB

9–1643

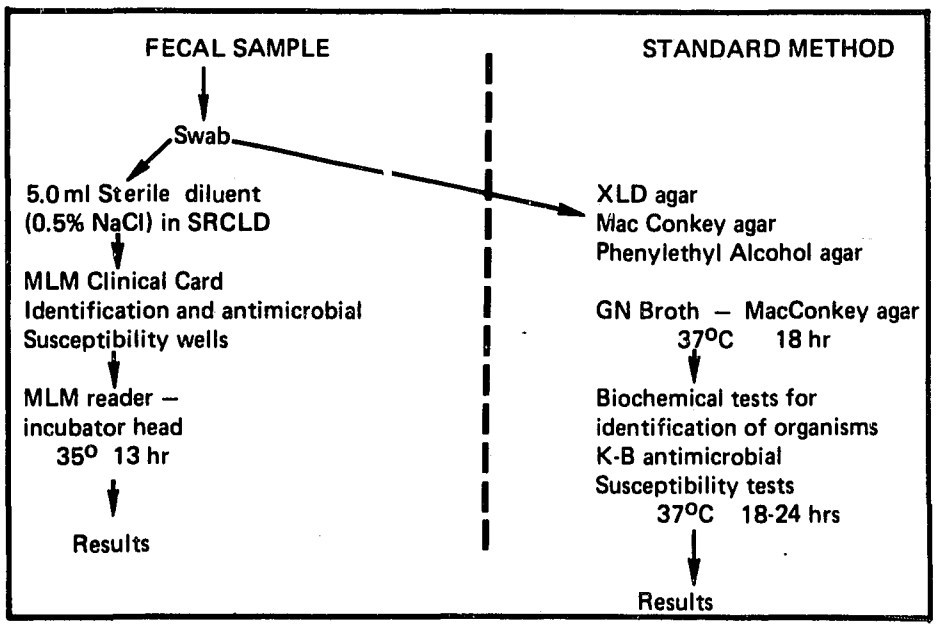


FIGURE 3–8
PROTOCOL FOR CLINICAL SAMPLES TESTS WITH FECES

grow in the presence of 6.5% NaCl and with the bile-esculin test. Yeasts were identified by germ tube formation and carbohydrate fermentation and assimilation tests. Conventional enumeration or counts were performed by spreading 0.1 ml amounts of serial 10-fold dilutions (in 0.5% NaCl) of urine samples on the surfaces of Blood agar and MacConkey agar plates, incubation at 35°C overnight, and performance of colony counts.

Seeded specimens were prepared by seeding clinical isolates in 0.5% NaCl solution for addition to filter-sterilized pooled urine samples. In the case of fecal samples, the test organism was added to a donated sample from a "healthy" individual. The sample was analyzed for microbial content prior to and following seeding.

3.1.8 Quality Control and Shelf Life Studies of MLM Cards and SRCLDs

3.1.8.1 Quality Control and Media Performance Testing - Quality control testing was performed on any large volume production (greater than 300) of MLM Cards. Additionally, records of quality control testing were obtained on various lots of McDonnell Douglas prepared media. The records of these identical formulations were used as a baseline performance check and evaluation for subsequently prepared MLM Cards.

The performance tests were evaluating MLM media with the same strains of organisms utilized by the MDC commercial venture. Tests were conducted as follows:

Preparation of Bacterial Seeded Samples

1. Twenty-four hours prior to the inoculation of the MLM Cards, cultures were streaked for isolation on 5% Blood Agar Plates and incubated 35-37°C overnight.
2. A turbid suspension of this overnight culture was prepared in sterile 0.5% NaCl. Turbidity was adjusted to match a 0.5 McFarland standard.
3. A 1:100 stock dilution of the suspension was prepared and used for filling the MLM Card, inoculating standard methods tests and for Kirby-Bauer sensitivity broth inoculation.
   a) 0.5 ml of the 1:100 stock dilution was added to 5.0 ml of 0.5% NaCl for MLM Card media tests.

b) 5 µl of the 1:100 stock dilution was added to 5.0 ml of 0.5% NaCl for the MLM enumeration test.

c) An aliquot of the stock dilution was further diluted 1:100, and 1:10; 0.1 ml of this final dilution was plated onto a blood agar plate and incubated overnight.

MLM Card Testing - The prepared samples (3a, 3b) were used to fill the MLM Cards. The Cards were incubated in the MLM reader incubator heads. For purposes of quality control testing, the results were noted for the 13-hour cycle but data was recorded to 15 hours.

3.1.8.2 Sterility Tests - Assembled SRCLDs - Sterility tests were conducted on assembled SRCLDs. Four units were tested from each of three groups. The three groups consisted of the following:

Group A) Assembled units - no electron beam sterilization

Group B) Assembled units - 2 mr sterilization

Group C) Assembled units - 3 mr sterilization

The purpose of this study was to determine the effectivness of electron beam sterilization for assembled SRCLD's.

3.1.8.3 Media Shelf-Life Tests - Prepared MLM Cards were tested for media efficiency following 3 months storage at 4°C. The media and antibiotics utilized in the MLM Card were identical to those used in the commercially prepared AMS Identi-pak with the exception of Acinetobacter-Herellea, Beta streptococcus, and Fungi media.

Shelf-life records were obtained on the commercially prepared media and are incorporated into this report in order to provide information concerning long-term shelf-life of the identical MLM media.

The same organisms used in the quality control testing of the prepared Cards were used in subsequent shelf-life studies.

Procedure for Card inoculation was the same as outlined under 3.1.8.1 Quality Control and Media Performance Testing.

3.2 HARDWARE AND SOFTWARE

Major items described in this section are as follows: (1) Cassette/Card; (2) Sample Loading Equipment; (3) Incubation and Detection Instrument; (4) Ancillary Equipment; and (5) Software. These items and their evolution is provided below.

3.2.1 Cassette/Card - Design of an easily built, trouble-free, biological sample processor has been a major challenge since the MLM conceptual design. Many designs were devised and tested for compatibility with the microbes, media, enumeration methods, and the optical densitometer technique used to detect growth. The cassette/Card has progressed, during the multiple phases of the program, from a simple two channel cassette to four channels, then five, then ten with combined cassettes for detection and sensitivity. Now a complete 60 channel Card with combined detection, antibiotic sensitivity, and enumeration for 12 different organisms has been developed from the previous work.

3.2.1.1 Serial Dilution Cassette - The first design for a serial dilution cassette is shown in Figure 3-9 and included four incubation and detection cuvettes and four serial filters. This design was compatible with the early two channel optical detection heads which used analog circuitry. The addition of a control channel for laboratory testing brought the detection port requirement to five for the filter cassettes.

A new five channel filter cassette design evolved which utilized milled grooves and drilled holes on only two surfaces (excluding input holes). The design, Figure 3-10, was straightforward for machining, and it required sealing tape application on only two surfaces. This improved the cassette sealing by eliminating tape peeling problems associated with the sealing of the narrow cassette edges. The new five channel design was functionally identical to the four channel filter cassette, except for the added control channel, and the only operational change was to load filters perpendicular, instead of parallel, to the cassette face.

3.2.1.2 Antibiotic Cassette - A requirement to provide five antibiotic sensitivity tests for a selected organism was handled by designing a combination cassette with five additional detection ports. These five antibiotic test wells were interconnected, as shown in Figure 3-11, to provide one pass of the inoculum through a filter prior to entry into each of the five antibiotic channels. The first

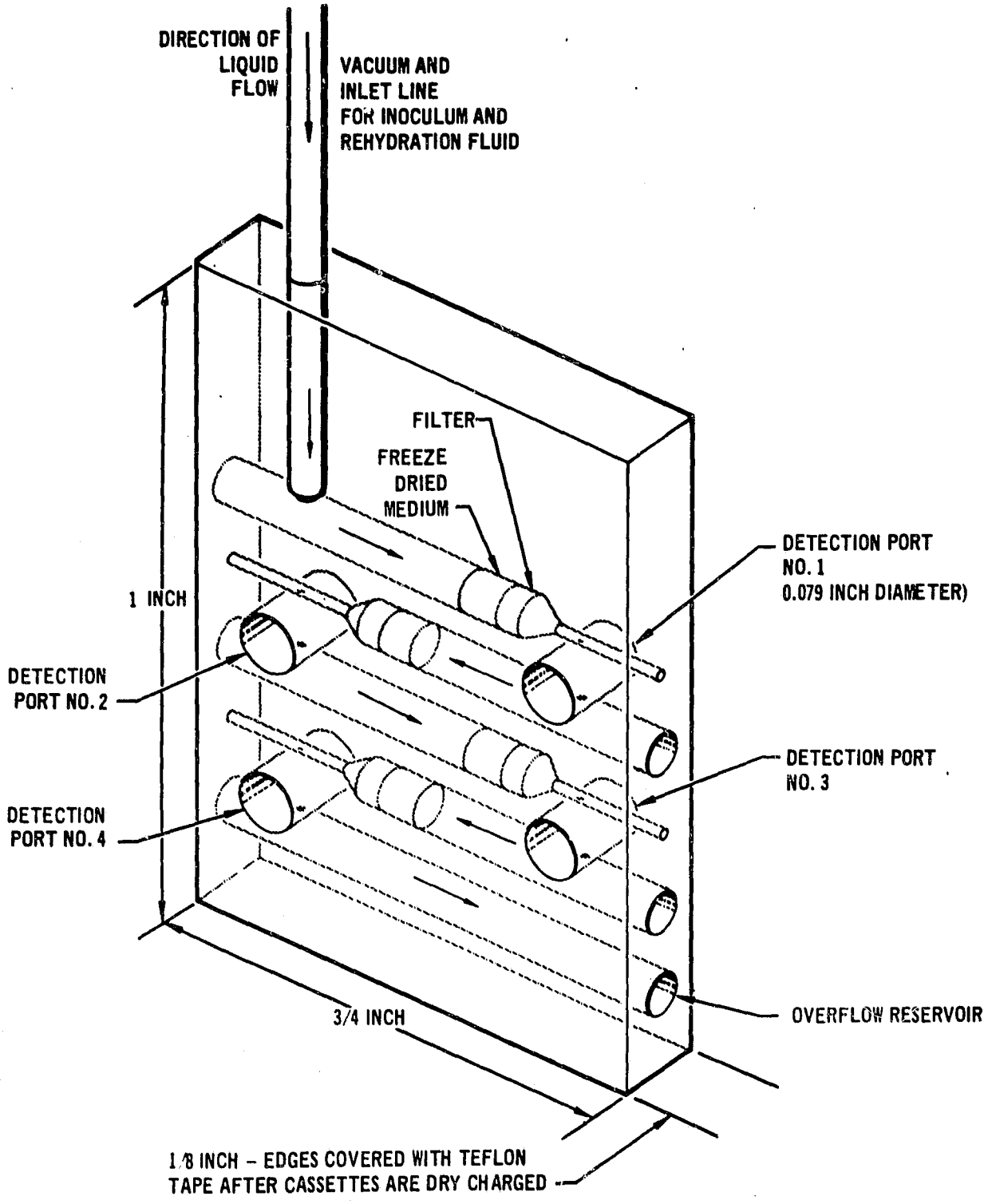


FIGURE 3–9
SERIAL DILUTION CASSETTE

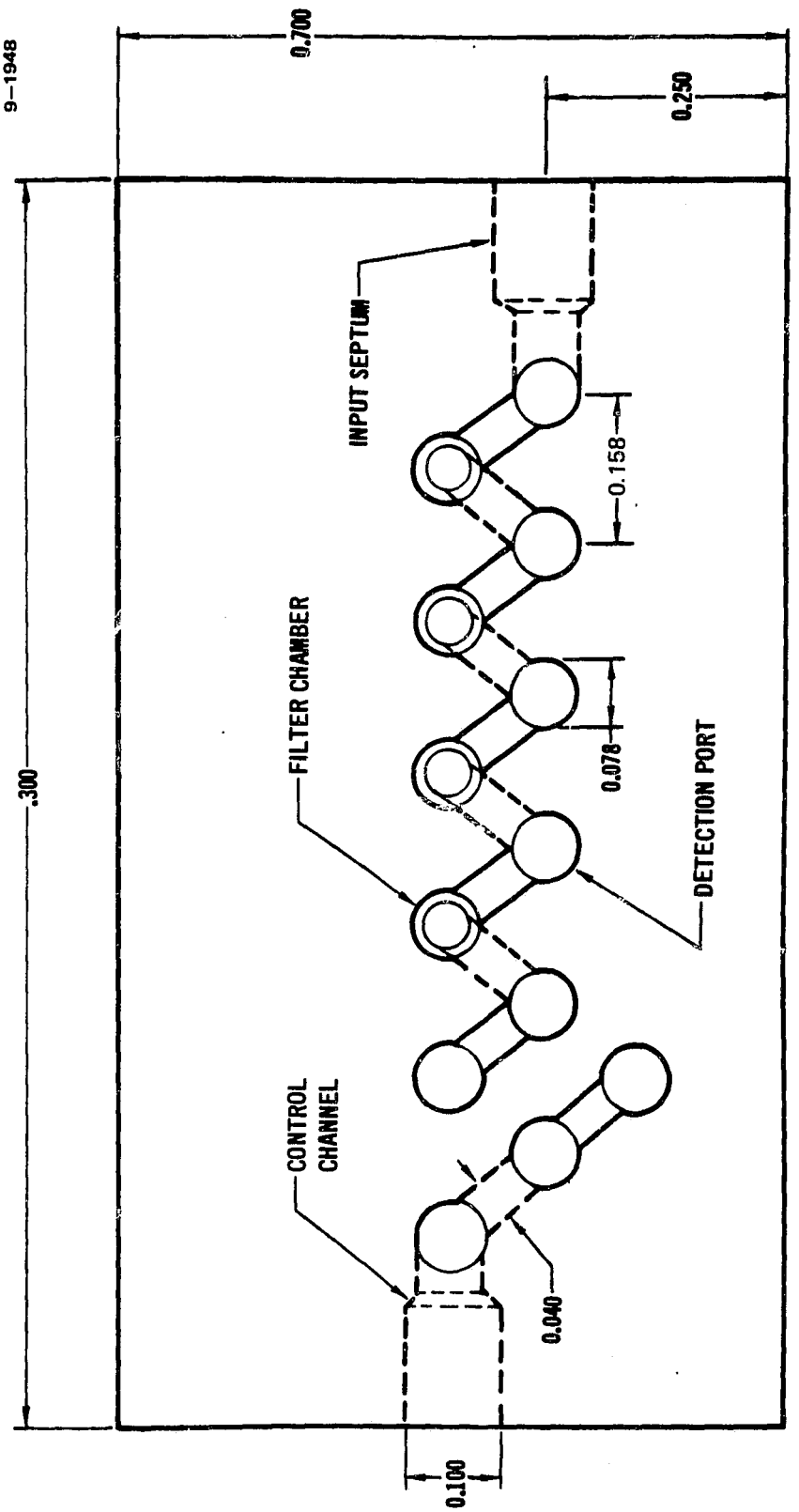


FIGURE 3-10
FIVE CHANNEL FILTER CASSETTE

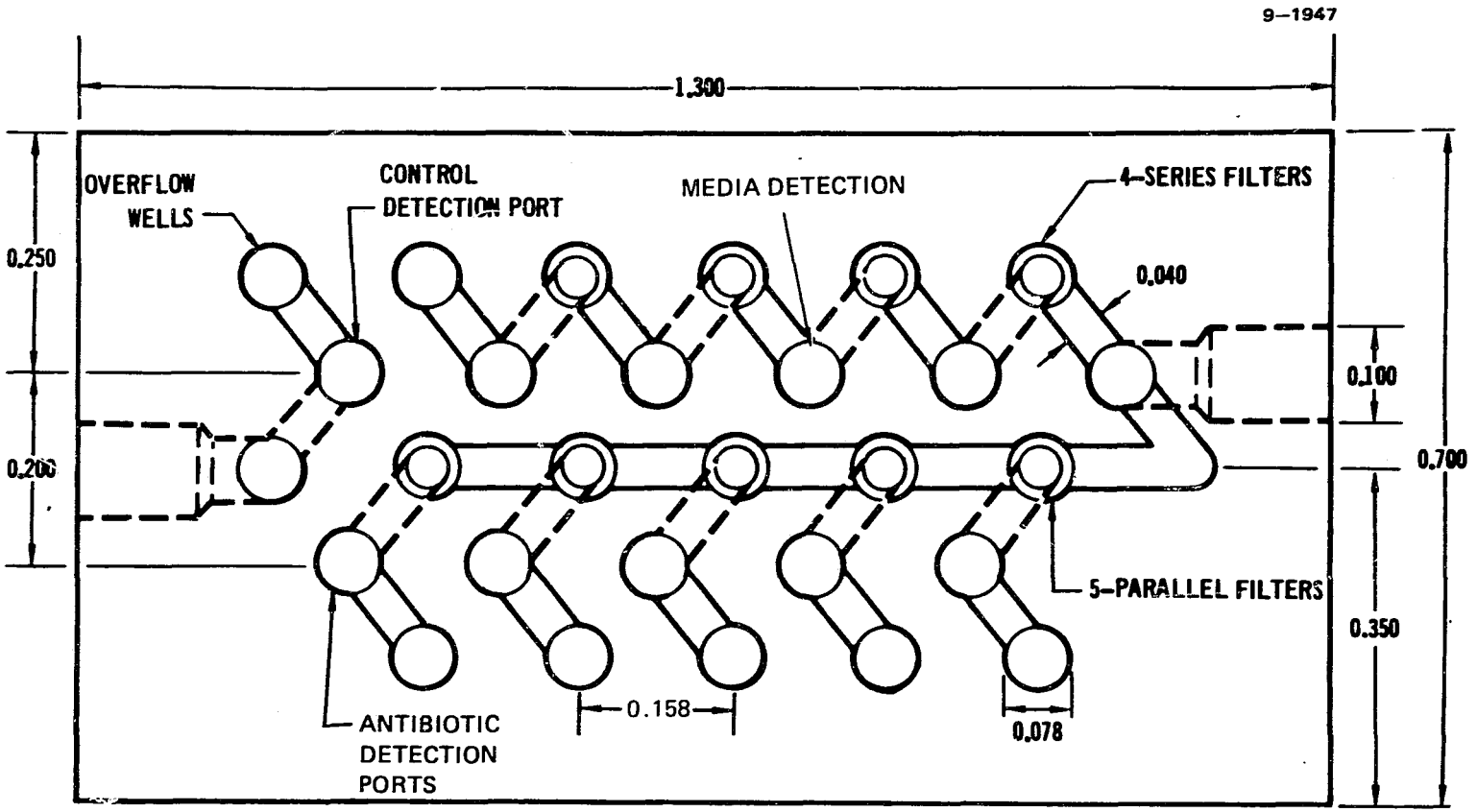


FIGURE 3–11
COMBINATION CASSETTE

detection port of the serial dilution chain (opposite half of cassette) follows a single filter stage and, therefore, serves as the inoculum control for the antibiotic sensitivity determination. This cassette design also includes a control detection port for uninoculated media.

Since each cassette to this point was used to test only one medium, an additional cassette design evolved and was produced in large quantity through an injection molding process. This unit, shown in Figure 3-12, is a dual filter cassette, combining two serial dilution labyrinths and corresponding controls on one plastic chip. Designed for compatibility with the combination (antibiotic) cassette, this dual cassette was used during the extensive reliability testing.

3.2.1.3 Integrated Cassette (Card) - With the concept of an arrangement of selective media for analyzing specimens successfully shown by thousands of data points taken demonstrating the feasibility and reliability, an effort was made to develop an integrated cassette (hereinafter called Card) containing up to 10 selective media, with five associated antibiotics, and filters. The Card design constraints included long term storage capability, compatibility with one g earth and null g space sample loading systems, and minimum loss of reliability from residual gas bubbles in the Card.

Several designs were evaluated. Working models of a few of the more promising designs were fabricated and tests were run to determine the feasibility of the design concept. The initial Card design, shown in Figure 3-13, was configured to be compatible with off the shelf emitter detector arrays and, therefore, allowed for 0.1 inch spacing between reading wells. The inoculum filling was accommodated by two independent manifolds provided to minimize incomplete filling of either side of the Card due to preferential fluid flow. The figure illustrates a urine sample Card with a serial dilution enumeration segment in the lower left hand corner. Triple redundancy was included in the reading well design to allow for automatic data analysis by majority voting. Section A-A view shows the serpentine path designed to flow the inoculum into all reading wells while minimizing trapped gas bubbles.

Two important considerations led the Card design away from this redundancy concept. First, additional diluent is required to fill the Card thereby reducing the sensitivity of the system and secondly, improved Card filling techniques and

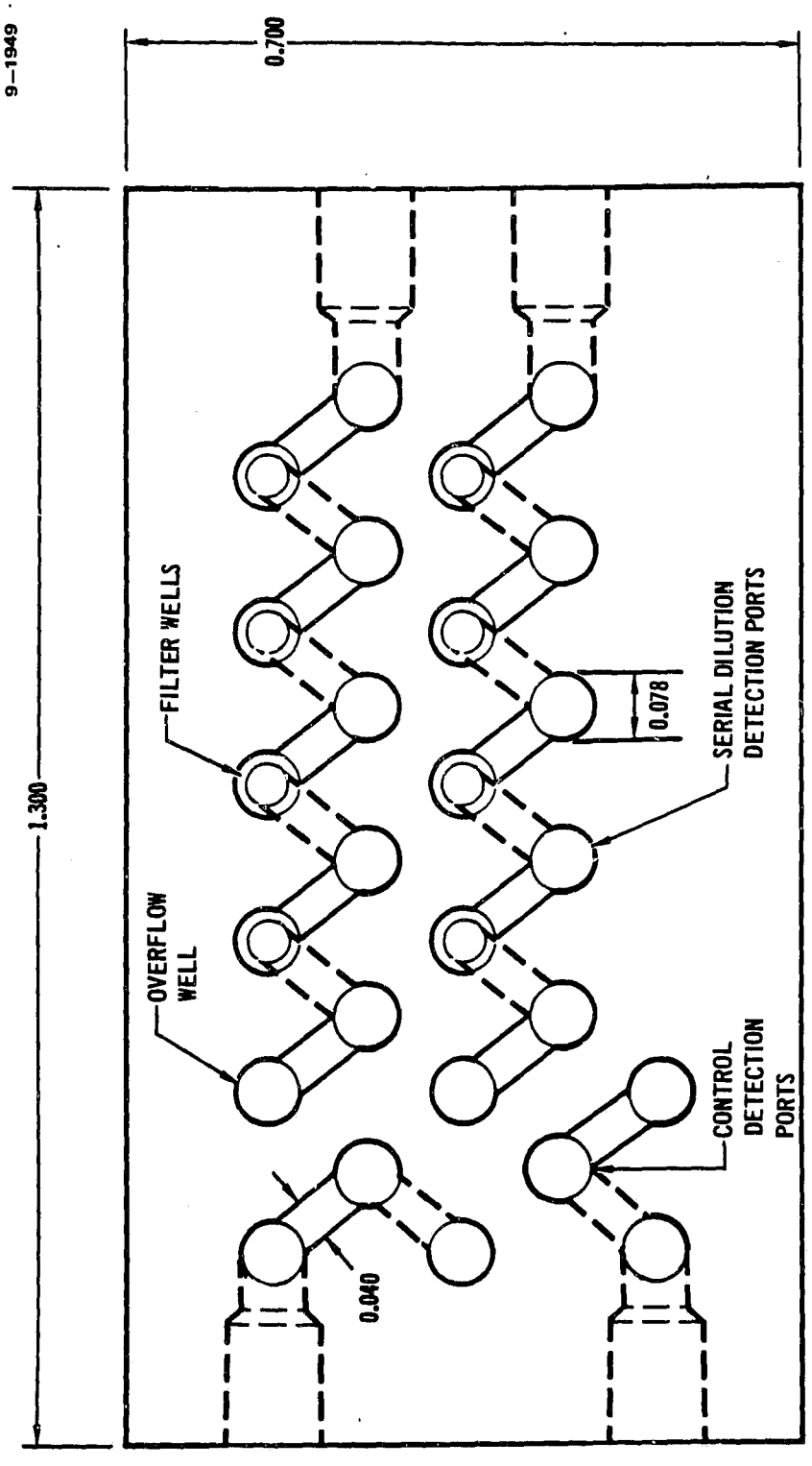


FIGURE 3-12
DUAL FILTER CASSETTE

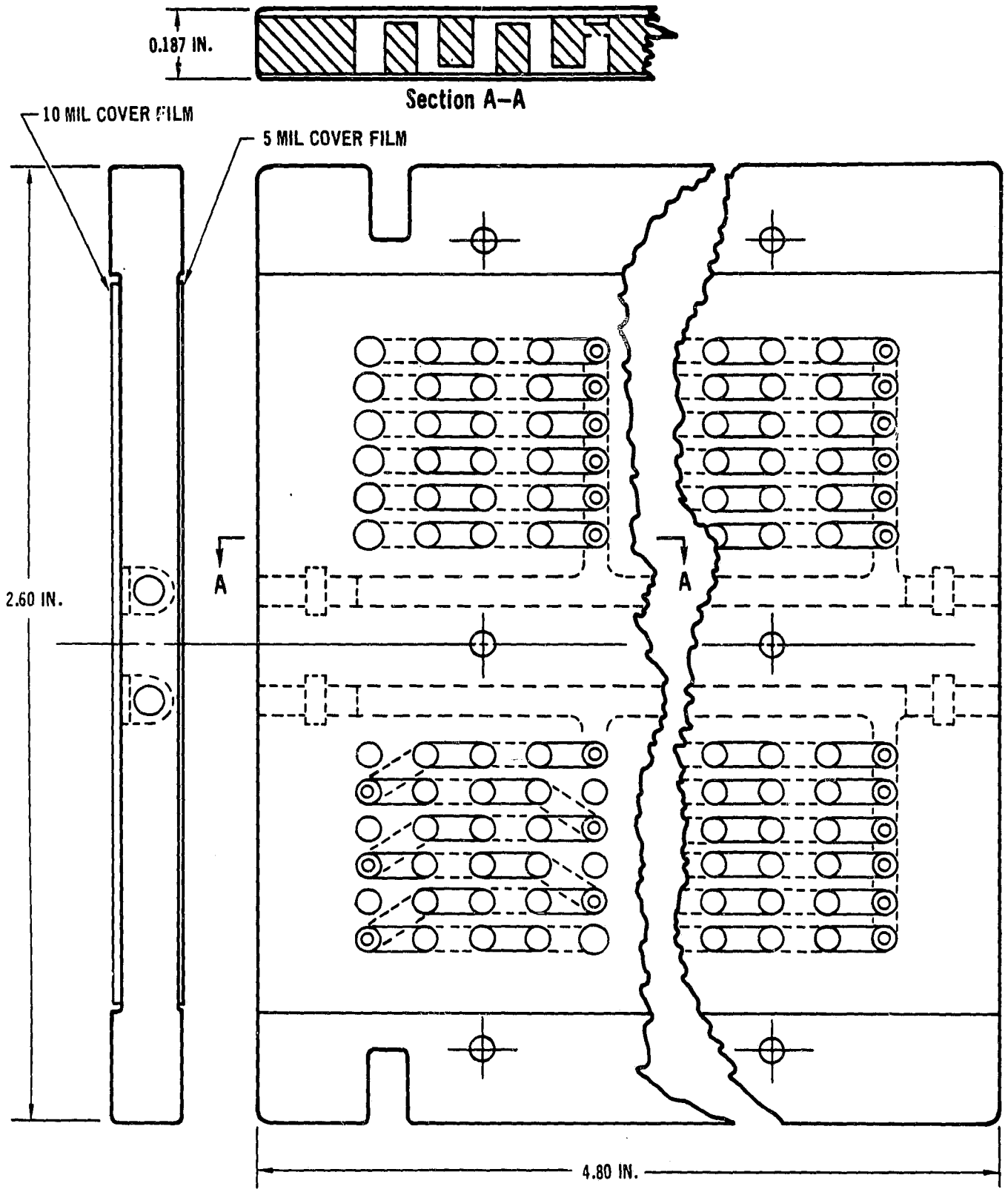


FIGURE 3–13
INTEGRATED CARD DESIGN

further exploration of the bubble causing factors minimized the bubble problem as a major cause of concern. The design shown in Figure 3-14 is the result. Well redundancy has been eliminated, the dual inoculum distribution channels have been replaced with a single centrally located distribution system, and overflow wells are reduced in size thereby reducing the inoculum volume required to fill the Card. This integrated Card design (urine) includes the capability for making nine microbial determinations with five associated antibiotics and determining relative number of microorganisms in the sample. During this study several Card designs were evaluated and their characteristics are compared in Table 3-5. Initially, the major concern was to minimize the quantity of inoculum required and a goal of 1 ml was

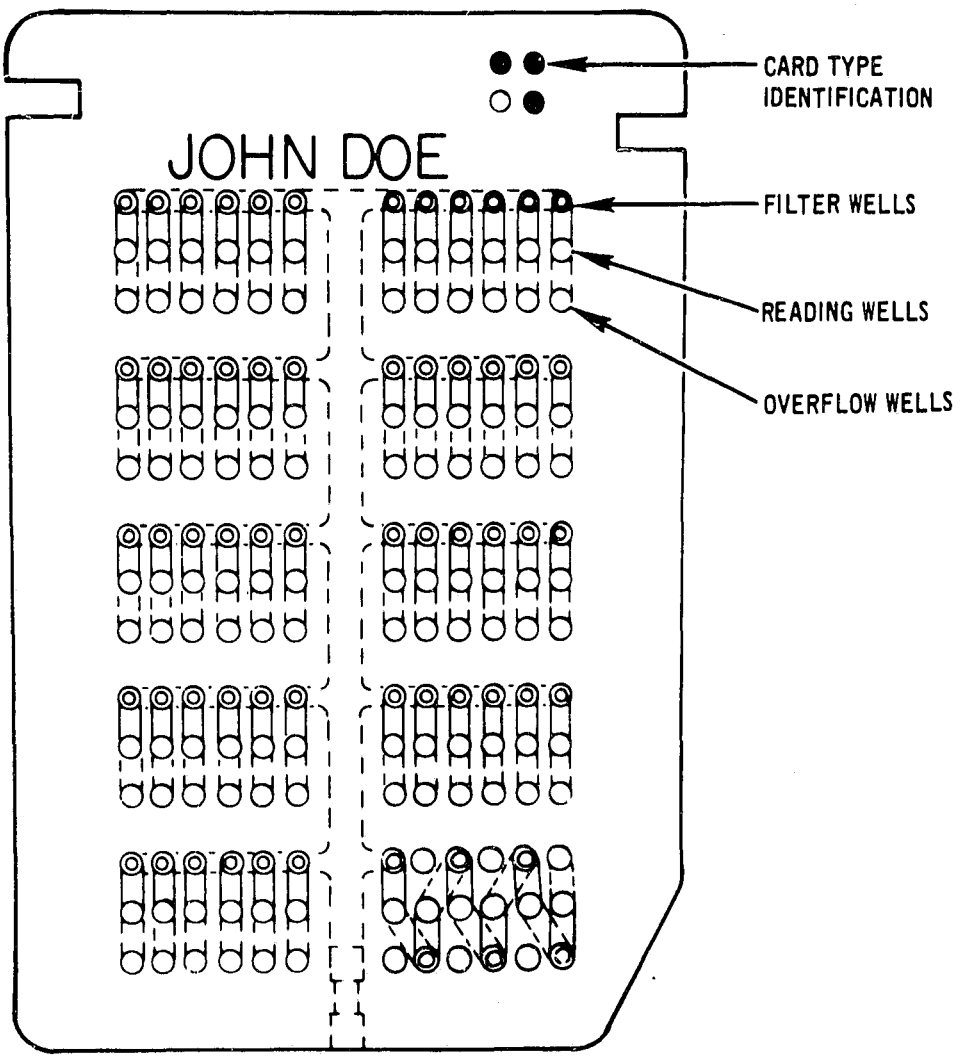


FIGURE 3–14
INTEGRATED CARD (URINE)

TABLE 3–5
PARAMETRIC COMPARISON OF CARD DESIGN

	SIZE	FLUID VOLUME	VIEWING WELL SIZE	SPACING BETWEEN VIEWING WELLS
1. TRIPLE REDUNDANT READING WELLS	2.60 IN. x 4.80 IN.	3.66 MILLILITER	0.062 IN DIA	0.100 IN.
2. ELONGATED READING WELLS (REDUNDANT READING)	2.00 IN. x 3.15 IN.	2.75 MILLILITER	0.062 IN.x 0.150 IN. SLOT	0.100 IN.
3. NO REDUNDANCY	2.00 IN. x 3.13 IN.	1.62 MILLILITER	0.062 IN. DIA	0.100 IN.
4. NO REDUNDANCY MINIMUM OVERFLOW	2.00 IN. x 3.13 IN.	1.30 MILLILITER	0.062 IN. DIA	0.100 IN.
5. ENLARGED READING WELLS AND MINIMUM OVERFLOW	2.20 IN. x 3.13 IN.	1.89 MILLILITER	0.080 IN. DIA	0.120 IN.

established to maximize the system's sensitivity. Reviewing the table shows that with no redundancy and minimum overflow well volume, the minimum fluid volume required to fill the Card is 1.3 ml.

One additional design was tested after loading problems became apparent during testing of ancillary Card loading equipment. The liquid media after loading tended to creep along the channels of the Card causing intermixing of the various media. Several solutions were considered which included increasing the size of the viewing wells and changing the manifold distribution system to reduce the likelihood of media creep into an adjacent media channel. A modified sector of a Card is shown in Figure 3-15. The increased channel length to each viewing well greatly reduces the possibility of adjacent channel intermixing.

Enumeration of microorganisms by the technique of using asbestos filters in the Card was demonstrated to be feasible when the cassettes are carefully hand loaded and packed. However, attempts to automate this technique were discouraging due to poor quality control. In addition, visual inspection of the test cassettes indicated that microorganisms concentrated on the filters frequently caused color changes several hours before those same changes occurred in the growth and detection wells which are viewed by the MLM. Putting the filters in the detection wells gave good but not accurate results due to the low light levels passed by the filter.

9–1853

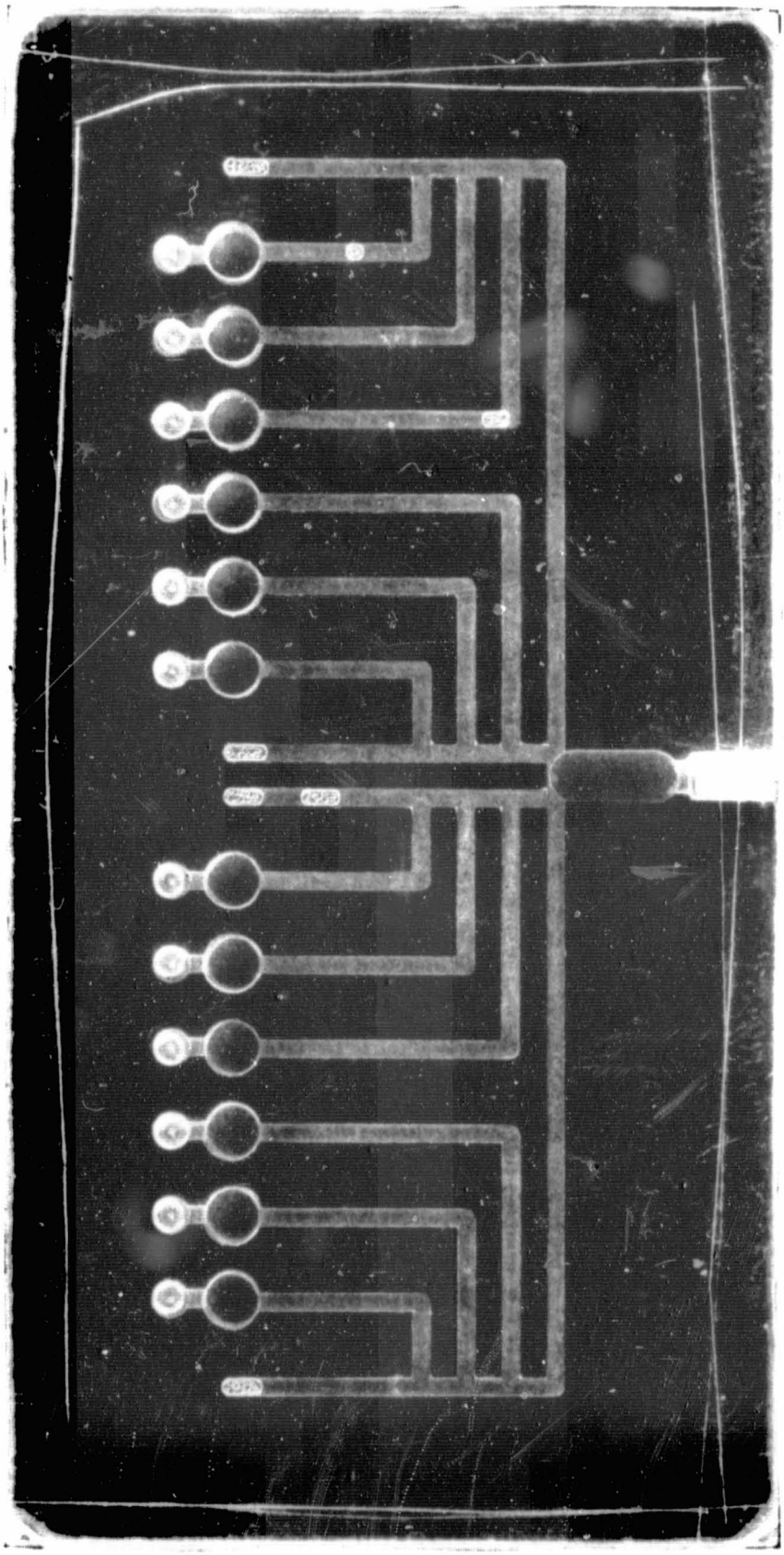


FIGURE 3–15
IMPROVED CARD SECTOR

The filter method then gave way to the method based on the Most Probable Number theory as described in Section 3.1.3, Current MLM Enumeration.

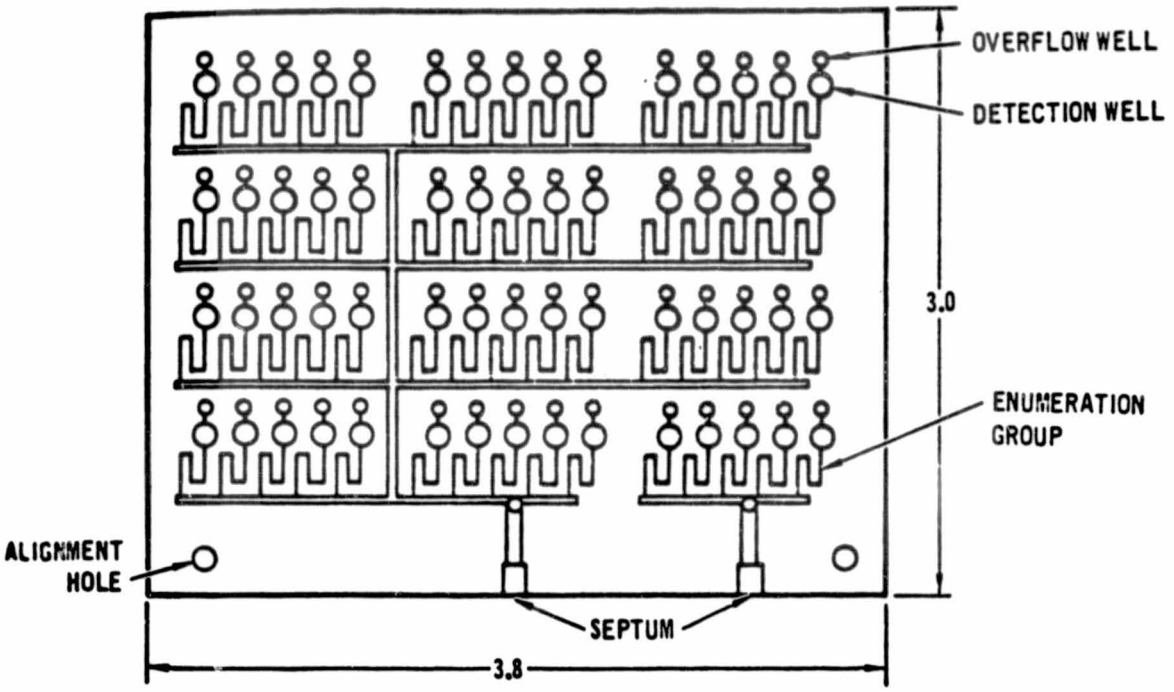
3.2.1.4 Clinical/Environmental Card - The Card design effort was then directed to a Card or Cards capable of using growth media, antibiotics and an enumeration method, when necessary, for use with either clinical or environmental samples. The enumeration method selected previously was the Most Probable Number (MPN). The initial requirements for the Cards are shown below.

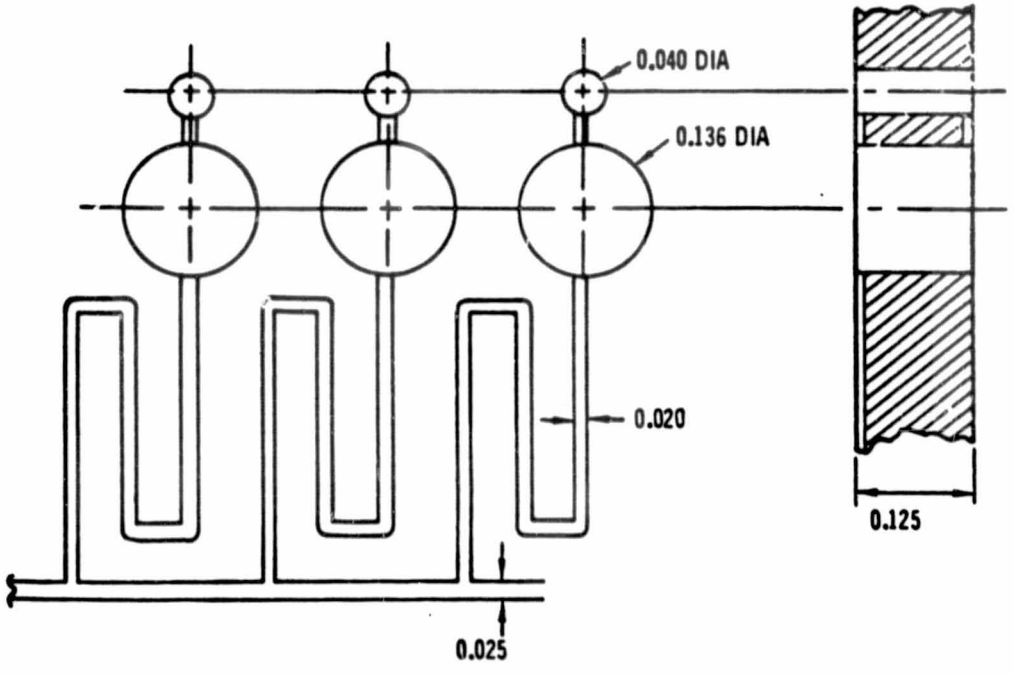
CLINICAL	ENVIRONMENTAL
1. Identification of organisms	1. Same as Clinical
2. Enumeration of organisms - (urine samples only)	2. Not applicable
3. Antibiotic susceptibility	3. Not applicable
4. Detection well allotment 5 enumeration; 1 positive control up to 9 media X6 (5 antibiotic and 1 growth)	4. Detection well allotment no enumeration; 1 positive control up to 9 media X1 (growth only)
TOTAL - 60 detection wells	TOTAL - 10 detection wells

Since the Environmental Card required fewer detection wells than the Clinical Card, it was concluded that an Environmental Card could be designed to accept more than one sample. Multiple environmental samples could then be incubated in the same space as one clinical sample assuming loading could be accomplished without problems.

The design of the two Cards is shown in Figures 3-16 and 3-17. Each Card has 60 detection wells in which inoculum diluent and media are combined, incubated, and examined optically for signs of growth. The Cards are identical except for the grouping of the detection wells.

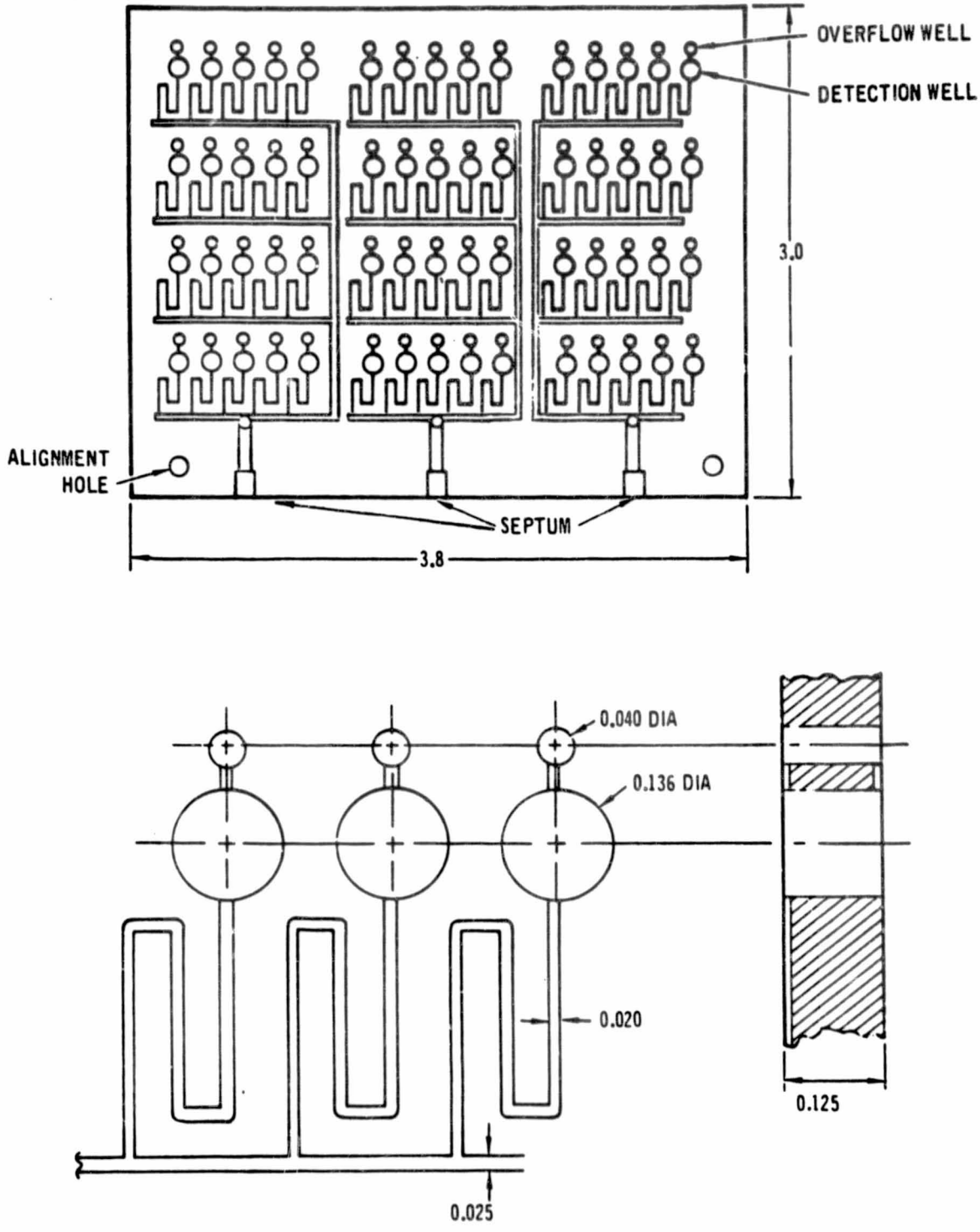
The Clinical Card has two separate groups of detection wells, Figure 3-16. The larger group of 55 detection wells is for organism identifications and antibiotic sensitivity tests. The small group of five wells receives a diluted inoculum for enumeration. Two loading devices are required to load the Clinical Card. The





DETECTION WELL VOLUME: 0.032 ML
CARD VOLUME: 2.08 ML
LABYRINTH DISTANCE BETWEEN ADJACENT WELLS: 2.04 IN.

FIGURE 3–16
CLINICAL CARD



DETECTION WELL VOLUME: 0.032 ML
CARD VOLUME: 2.08 ML
LABYRINTH DISTANCE BETWEEN ADJACENT WELLS: 2.04 IN.

FIGURE 3–17
ENVIRONMENTAL CARD

labyrinth like passageway between each detection well and the feeder channels serve to reduce the chance of contaminating diffusion occurring between adjacent detection wells. The overflow hole next to each detection well receives the residual air remaining in the Card after evacuation. The Card is covered on both sides with FEP Teflon tape which has silicone adhesive. The septum holes are sealed with silicone rubber.

The Environmental Card is similar except that the detection wells are grouped into three equal sets, Figure 3-17. This Card will accept up to three environmental samples and perform identification of each specimen. The Card may be used for less than three specimens if the cover tape is punctured in each unused segment. If the tape is not punctured in the unused areas, the expansion of the trapped air during the evacuation phase of the filling process will blow off the tape. Both Cards are prepared with freeze dried media.

Configurations of media within the Cards other than that given above are possible. In fact, the final configuration used is different. The Clinical Card includes 10 media with 4 antibiotics each, one single medium, one positive control and 5 enumeration for a total of 57 wells used. The Environmental Card includes 12 media and one positive control for each 20 well group. The listing of each Card is presented elsewhere.

3.2.2 Sample Loading Equipment - Several alternate concepts have been reviewed, during the course of the MLM Program, for handling swab acquired microbial samples. The basic concept of introducing the inoculum swab into a sealable packet or compartment containing the prepackaged diluent was the baseline for a loading device design developed during Phase II. This design is shown in Figure 3-18. A photograph of a two cassette test model is shown in Figure 3-19. Functionally, the swab is placed into the sealable packet containing a bag of diluent. After sealing the packet a vacuum is pulled on the entire system thus reducing introduction of bubbles into the cassettes. Rupturing the diluent bag allows the swab and diluent to be thoroughly mixed by digitation. Following mixing the transfer valve actuation automatically transfers the inoculum into the attached cassettes.

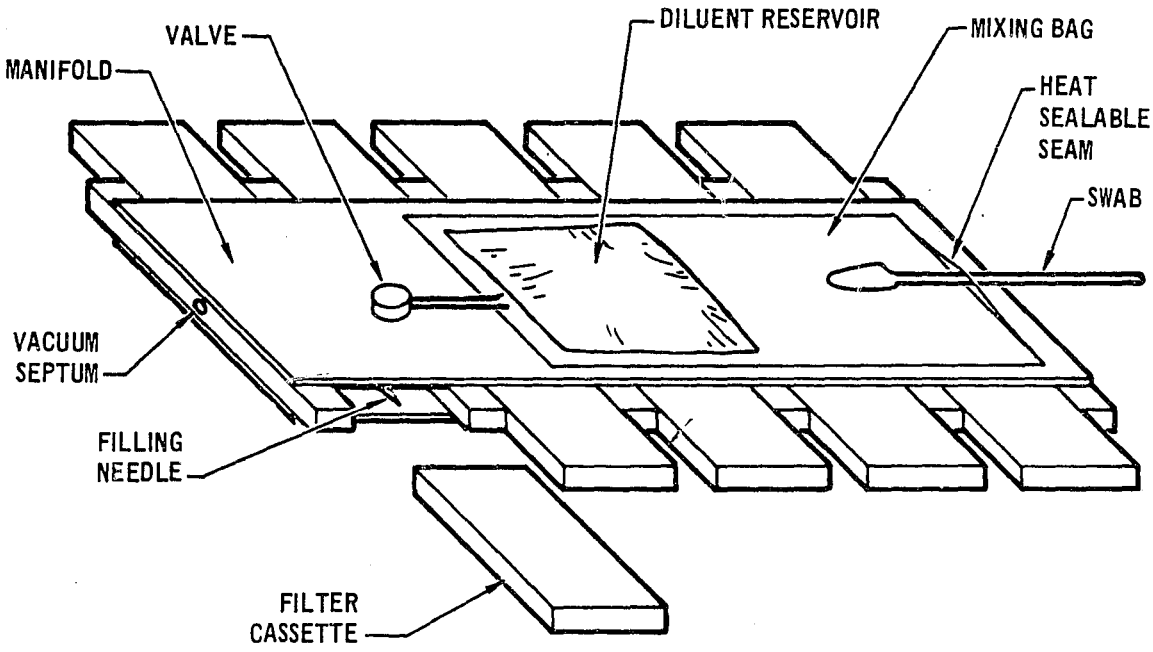


FIGURE 3–18
SEALED PACKET SAMPLE LOADING DEVICE

Results of tests using this design were varied. When care was taken to avoid excessive air mixed with the inoculum, the filling of the cassettes was virtually bubble free. However, leaks which subsequently developed around the mixing bag/transfer valve seal and in the manifold needle interface reduced the reliability of the testing results. The tests did indicate the feasibility of the technique, but also indicated the need for an improved concept which would allow easier reuse of the device, for continued laboratory testing.

Subsequently, a second inoculum handling device was designed which was intended to reduce the fabrication complexity by allowing use of injection molding. The design was functionally identical to that previously described. A model of this unit is shown in Figure 3-20. The diluent bag was replaced by a diluent reservoir compartment. The swab, introduced through an air lock to minimize bubbles, is pushed into the diluent compartment where mixing occurs by physical agitation. A transfer valve located in the center of the diluent compartment is activated after evacuation of the cassettes and adjoining manifold. The inoculum is transferred through the valve, filling the passages of all attending cassettes. During testing, several problems beleagured this design including: (a) complexity of initial assembly

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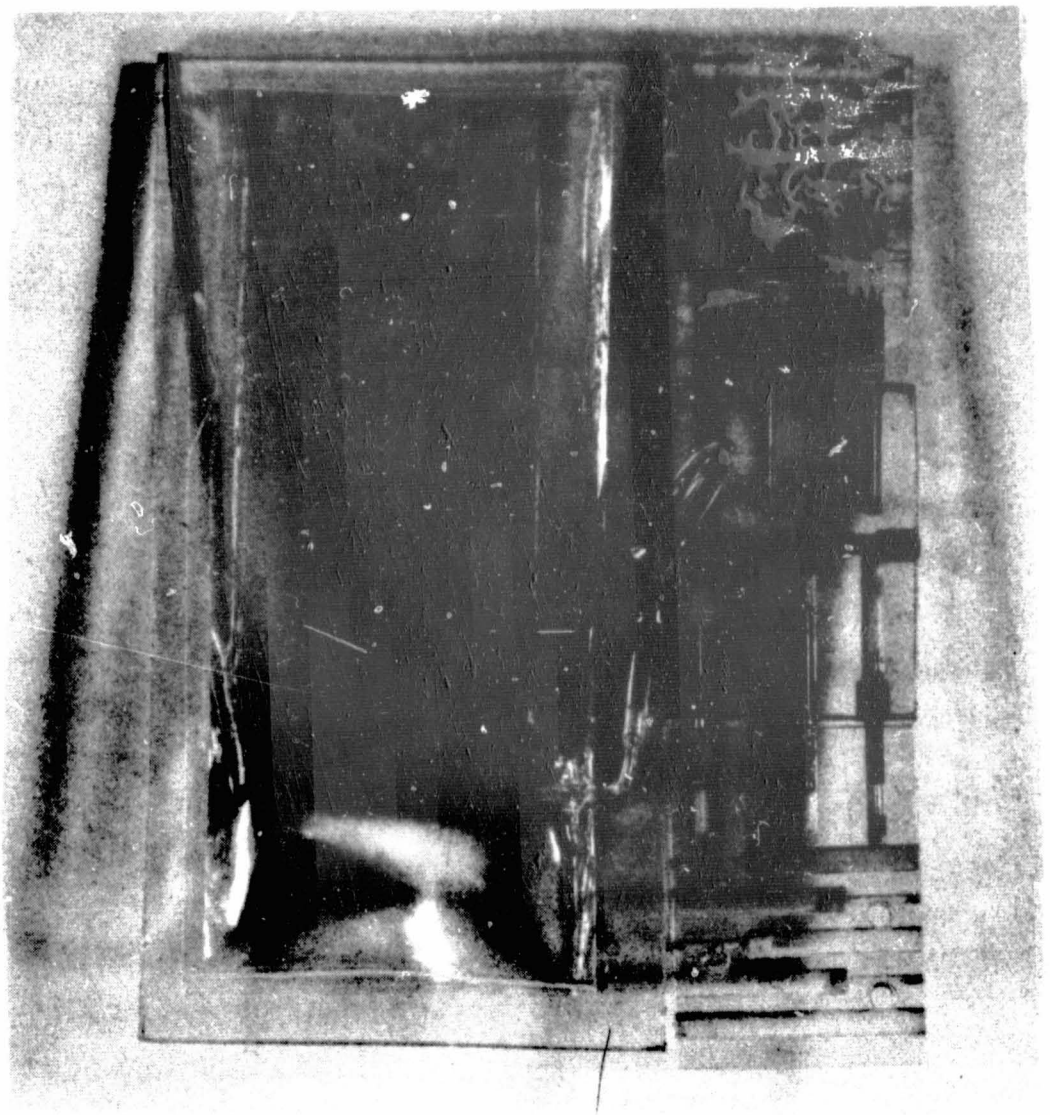

FIGURE 3–19
TEST MODEL
SEALED PACKET SAMPLE LOADING DEVICE

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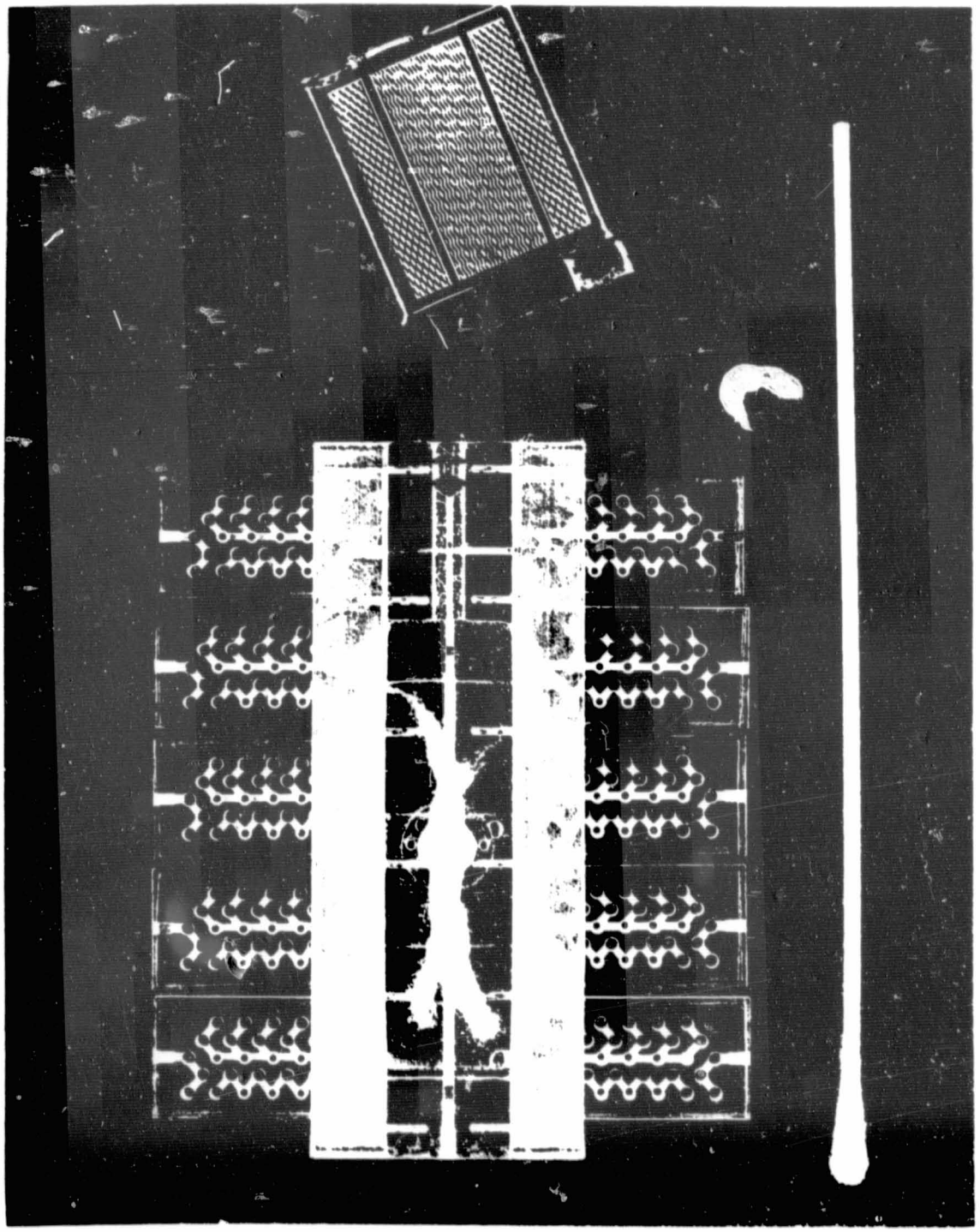

FIGURE 3-20
REFINED SEALED PACKET SAMPLE LOADING DEVICE

and reassembly after each test series, (b) myriad of small leaks around transfer needles, (c) fragility of the design ending in near destruction of the model during testing, and (d) reduced mixing convenience.

At this point, it became apparent that to achieve reliable results with the MLM, the cassette and its loading device must work well together. The combined units were now called the sample processor system (SPS) thereby emphasizing the importance of their interaction. The loading became more than a device for handling the inoculum and acquired the title of Sample Loading System.

The Sample Loading System (SLS) provides the interface between the sample acquisition device (swab for space applications, and either swab or pipette for earth based systems) and the sample growth and detection cassette or integrated Card. A vacuum system is used to evacuate the cassette/Card and filling device for bubble-free filling.

Early laboratory versions of the SLS implemented this vacuum filling technique by utilizing gravitational effects which allowed the cassette gases to be bubbled through a diluent chamber. The process is demonstrated pictorially in Figure 3-21. During evacuation, cassettes and inoculum laden diluent are degassed. After an appropriate evacuation period, the system is repressurized to ambient pressure allowing the inoculum to fill the cassette chambers. After the contract change notice redirecting the development effort to a high volume, land based application, an improved laboratory SLS was designed and fabricated. This system coupled with a disposable diluent cartridge, minimized the problems associated with the original the vacuum source to be regulated for slow initial evacuation to eliminate bubbling several cassettes are shown in the photograph of Figure 3-22. This combination was used successfully for filling several hundred cassettes during the antibiotic sensitivity testing and evaluation series. The controls in the filling head allow the vacuum source to be regulated for slow initial filling to eliminate bubbling up through the cartridge interface stack and for full evacuation (fast) to provide maximum elimination of air from the cassette. A second control provided for switching from evacuation to the filling mode. After approximately one minute evacuation time, this switch was placed in the fill mode and sample loading of the cassette was completed. A multi-station filling system is shown by the photograph in Figure 3-23. Using this multi-station filling system, 50 cassettes can be filled in

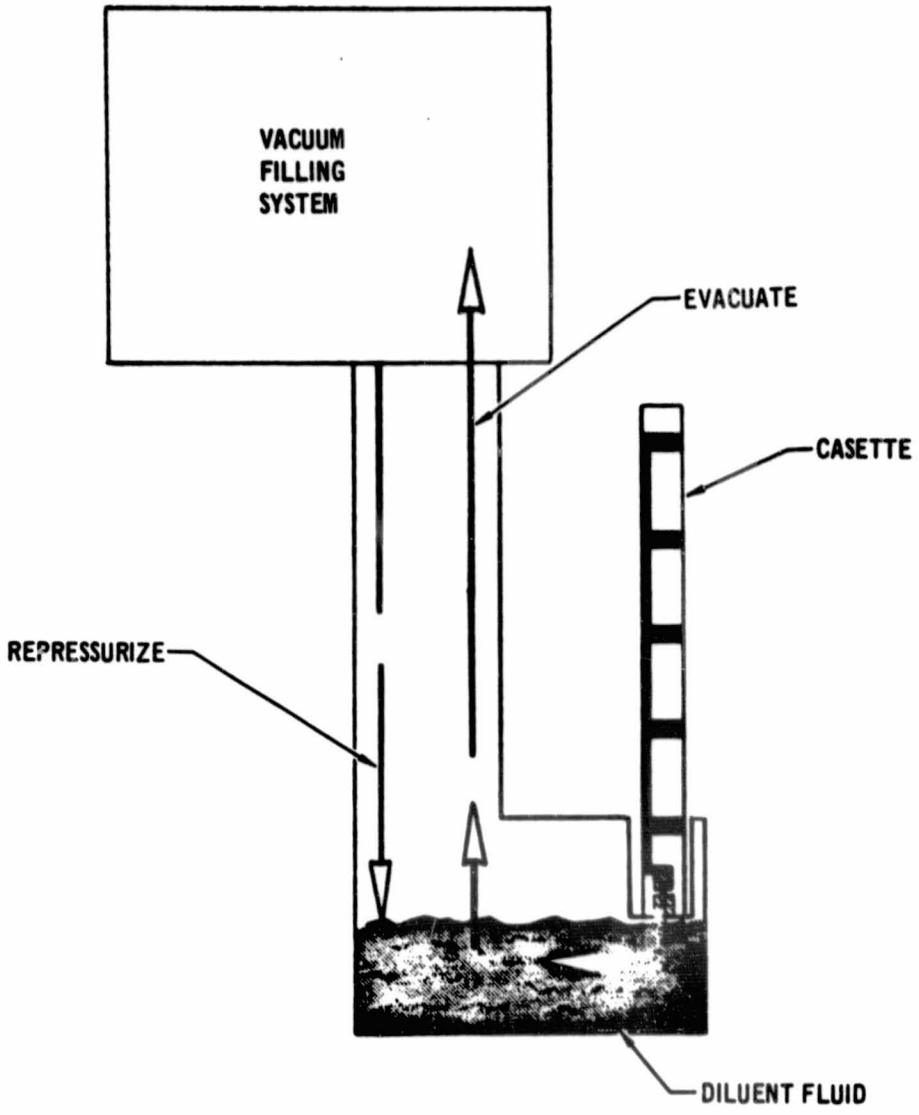

FIGURE 3-21
SAMPLE LOADING SYSTEM CONCEPT

approximately 15 minutes. This offered a substantial improvement over the technique used during the previous contract phase which required nearly an hour for sample loading. This unit has a slight interaction if filling and evacuation is attempted simultaneously. This unit performed successfully during spacelab mockup tests at NASA, JSC.

This design of the sample loading system was compatible with the integrated Card requiring only a minor modification to the disposable diluent cartridge. A photograph of a modified cartridge is shown in Figure 3-24. An improved diluent

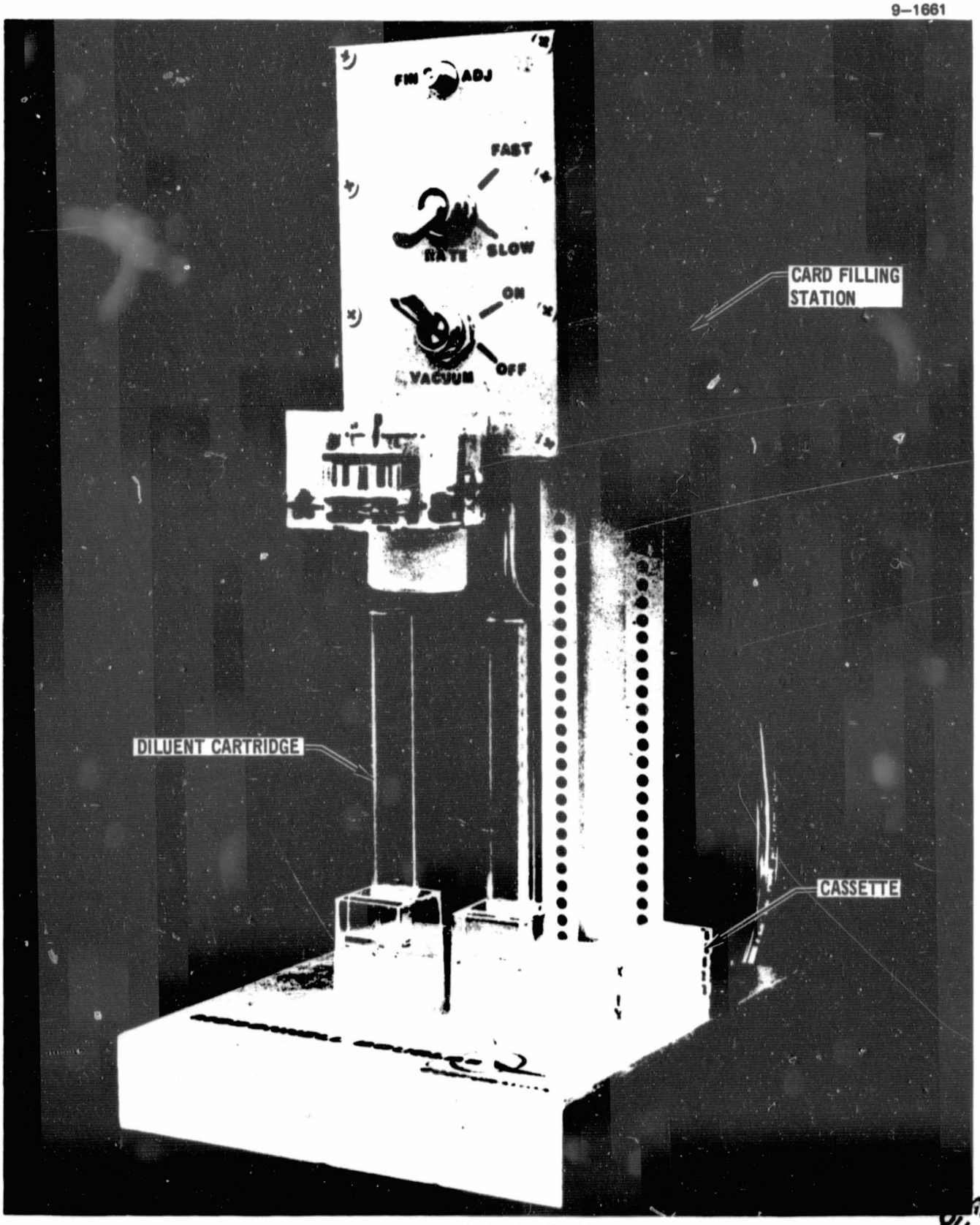

FIGURE 3–22
SAMPLE LOADING SYSTEM

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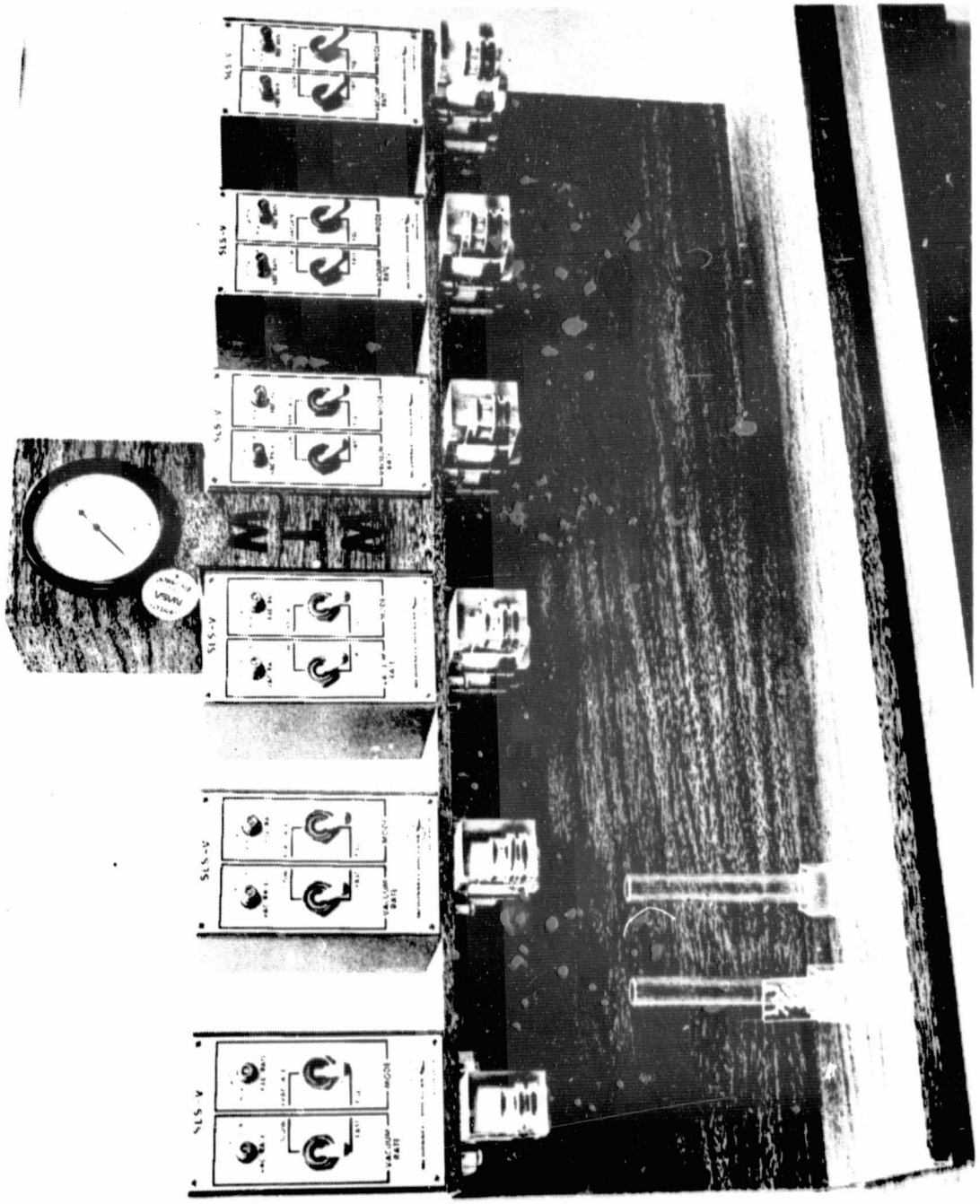

FIGURE 3-23
MULTI STATION CARD FILLING SYSTEM

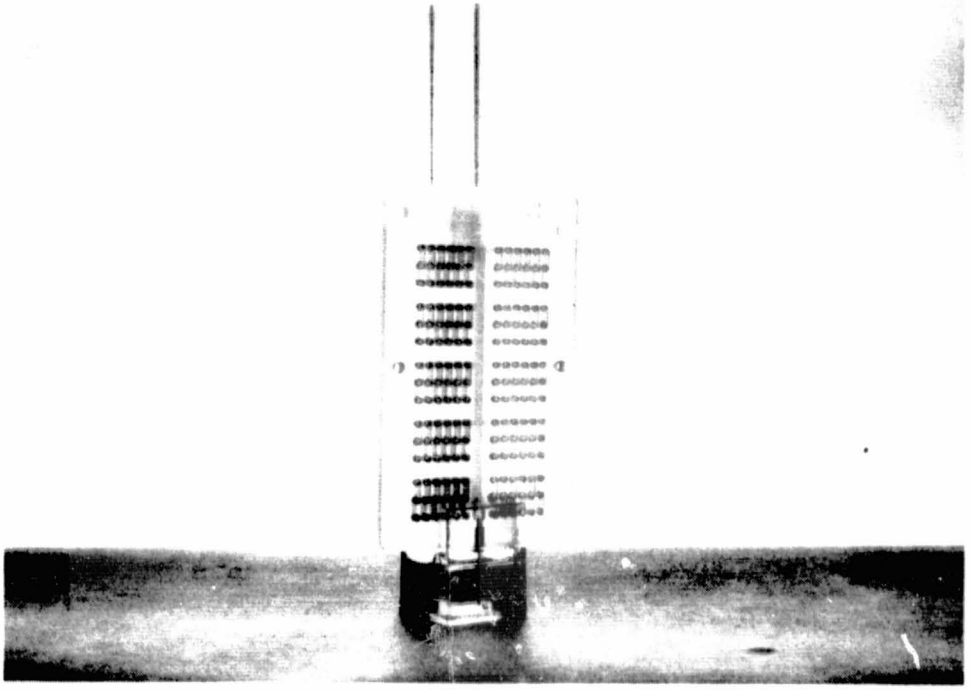

FIGURE 3–24
MODIFIED DILUENT CARTRIDGE

cartridge was designed which minimizes the inoculum fluid volume required for proper Card filling and greatly reduces the migration of inoculum bubbles which have been seen to travel up the diluent cartridge evacuation stack during the filling process. Migration of these inoculum bubbles up the evacuation stack has caused concern for two reasons. One, these bubbles are potential pathogen contaminants of the filling system and two, when the system is protected from contamination by absorbent filters in the air pathway, bubbling inoculum fluid could be lost to these filters thereby reducing the probability of a successful Card fill. The drawing in Figure 3-25 shows the details of the cartridge design including the throw-away contamination filter interface between the evacuation stack and the Card filling system, and illustrates the bubble expansion step in the evacuation stack which breaks up the bubbles before they could travel any substantial distance up the stack. The diluent fluid chamber was rearranged to allow nearly all of the inoculum to be transferred into the Card during the filling cycle. A photograph of a mockup of this proposed design is shown in Figure 3-26.

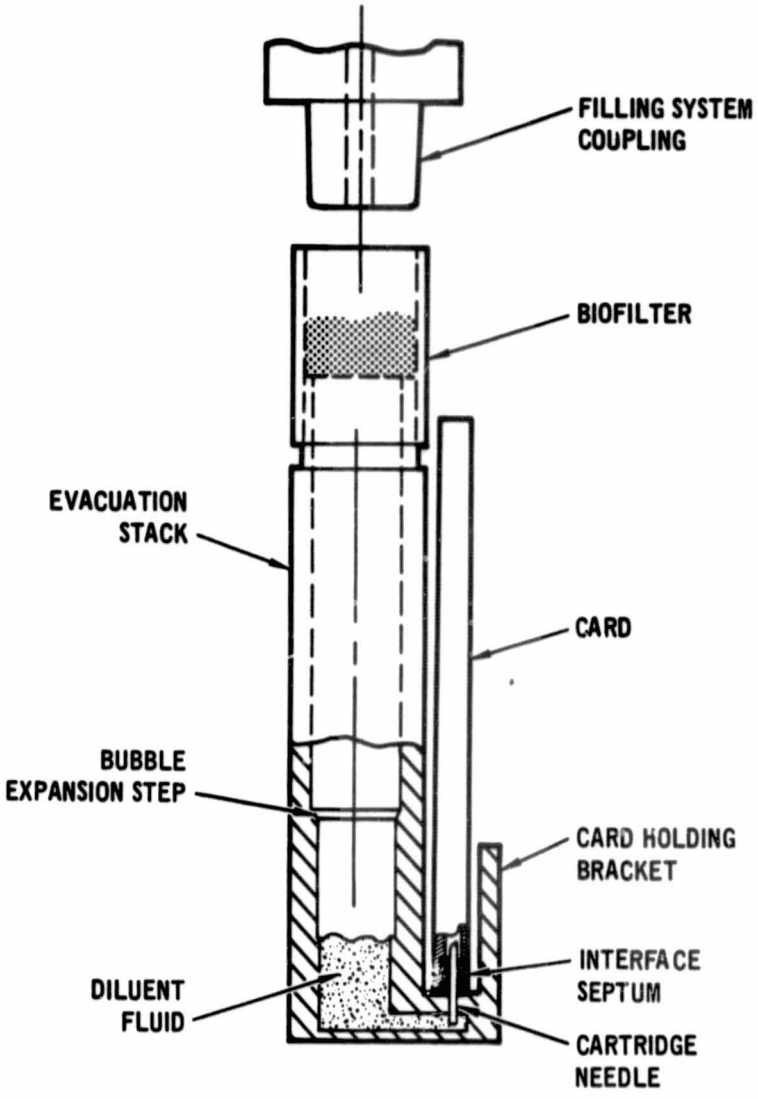

FIGURE 3–25
CARD-CARTRIDGE INTERFACE DETAIL

Several Cards were fabricated and subjected to tests under various conditions. Specific areas of interest included: (a) evacuation time, (b) vacuum level (MMHG), and (c) filling rate. Table 3-6 summarizes the filling success obtained under these conditions. This Card filling study provided a means for selecting optimum evacuation times, vacuum levels, and filling rates. Other parameters associated with the Sample Loading System were also varied in an effort to determine a technique for eliminating the appearance of bubbles during incubation. Several techniques were tried including venting of the Card after filling, preheating of the diluent to the incubation temperature and overpressurizing the diluent cartridge to increase the pressure equalization within the Card. The first and last techniques were found to

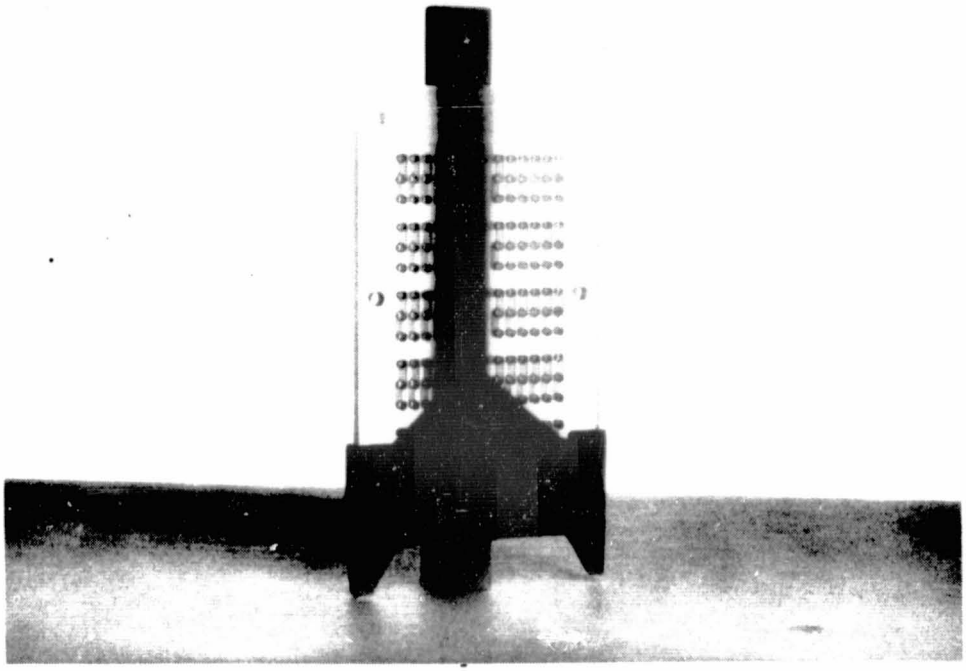

FIGURE 3–26
IMPROVED DILUENT CARTRIDGE

TABLE 3–6
CARD FILLING STUDY

CARD FILLING PARAMETER		% SUCCESS
1. EVACUATION TIME		
1 MINUTE		75%
5 MINUTES		97%
10 MINUTES		98%
2. VACUUM LEVEL		
25 MM Hg		80%
15 MM Hg		95%
5 MM Hg		99%
3. FILLING RATE		
(SLOW)	0.027 ML/SEC	90%
(MEDIUM)	0.081 ML/SEC	99%
(FAST)	0.81 ML/SEC	95%

greatly reduce the appearance of the bubbles, however, the first technique was deemed impractical due to the compromise of the integrity of the sealed Card environment. The Card filling sequence was modified to include a 6 psig overpressurization to the diluent cartridge. Extensive testing of this filling sequence has shown consistent improvement in the reduction of bubbles appearing after prolonged incubation periods. However, there was some indication from limited testing that the overpressure causes an oil can effect of the tape membrane covering the viewing well, thereby distorting it in the reverse direction. A filling method which does not stress the tape membrane in either direction at any time during the filling process would seem to be ideal.

3.2.2.1 Null-G Sample Loading System - At this time, the development of the MLM changed to that of a flight prototype. Two Null-g filling station concepts were studied. Centrifugal force was used in both concepts to separate the diluent fluid from the gas. Both systems are enclosed in a vacuum chamber to minimize gas diffusion through the cover tape resulting in smaller residual gas bubbles in each overflow well. Drawing a vacuum on both sides of the cover tape avoids most tape distortion during the filling process by avoiding a large pressure differential across the cover tape.

Both diluent cartridges had similar sample entry ports, with both a solid sample port and a fluid sample port. The solid sample port consists of inner and outer plugs and, in essence, forms an airlock. The fluid sample port is a septum through which the fluid sample can be injected.

3.2.2.2 SLS Concept A - In Concept A, Figure 3-27, the diluent cartridge rotates while the Card is stationary. After the sample has been mixed with the diluent in the cartridge, the cartridge is placed into the vacuum chamber. An adapter needle is inserted into the selected Card. This arrangement is then placed into the vacuum chamber next to the diluent cartridge. The driver assembly, which spins the cartridge, is advanced onto and engages the end of the diluent cartridge. During this action the vacuum tube is inserted into the specimen airlock plug opening the airlock valve. Vacuum is applied after the spinning cartridge reaches sufficient speed to separate fluid and gas.

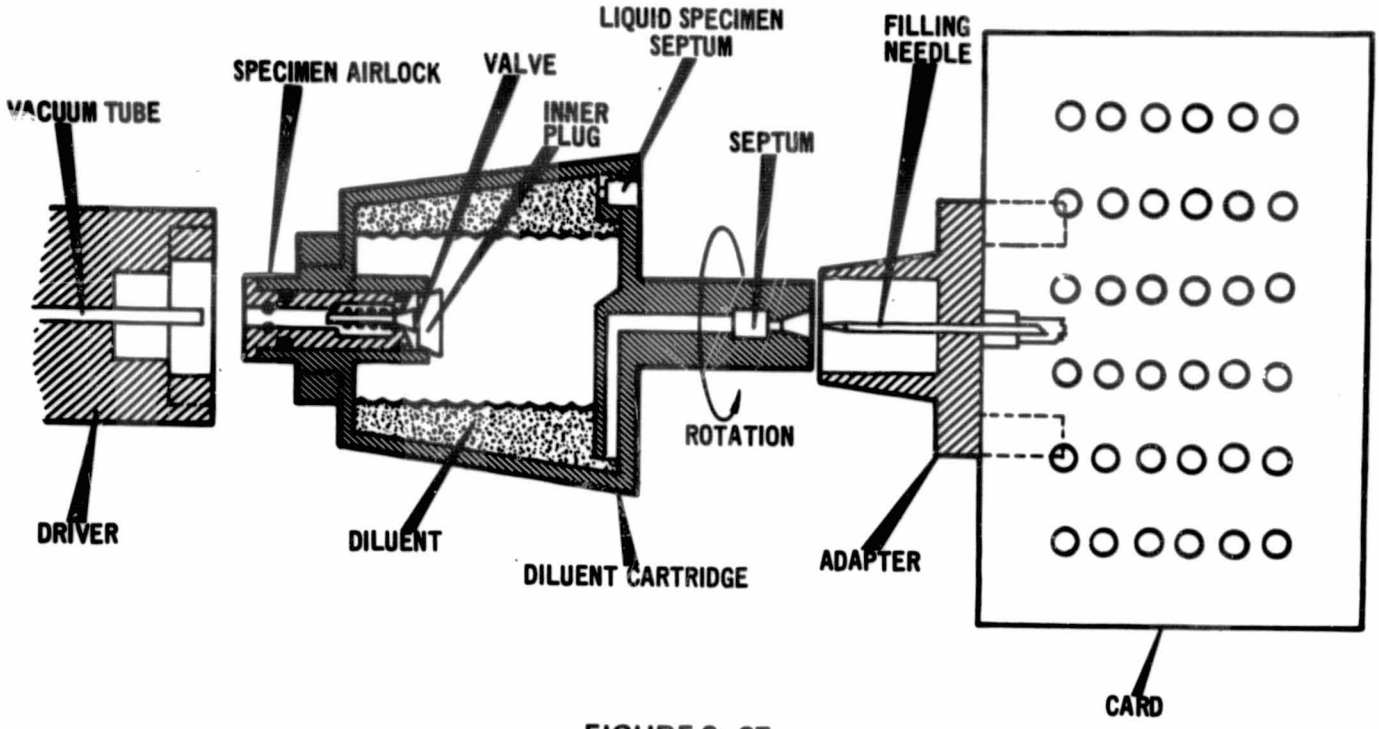

FIGURE 3–27
ZERO GRAVITY FILLING SYSTEM
Concept A

While the cartridge is being evacuated through the vacuum tube, the Card is being evacuated into the chamber through the adapter. When the vacuum chamber reaches its low vacuum level, the Card with adapter is advanced onto the spinning diluent cartridge causing the filling needle to penetrate the septum. The Card is filled by simultaneously raising the pressure in the diluent cartridge and the chamber at a controlled rate. When filling is complete, the Card, adapter, driver, and diluent cartridge are manually separated and the Card placed into the reading station. Movements of the Card and driver unit within the vacuum chamber are accomplished with pneumatic cylinders and/or servo motors.

Integrated Cards with 20 wells have successfully filled with this system. The residual air volume in the overflow holes was much smaller than achieved with previous sample loading systems. The air volume also has shown less tendency to expand during incubation.

3.2.2.3 SLS Concept B - The difficulty of filling a Card through two septums with two or three separate dilutions utilizing Concept A and the questioned reliability of the simple rotating seals led to the design of SLS Concept B. With

Concept B, Figure 3-28, the SLS may use from one to three diluent cartridges to simultaneously fill the Card. Both a Card and the necessary cartridges are mounted on a rotating turntable, which is enclosed in the vacuum chamber. The diluent cartridge used in Concept B is shown in Detail in Figure 3-29.

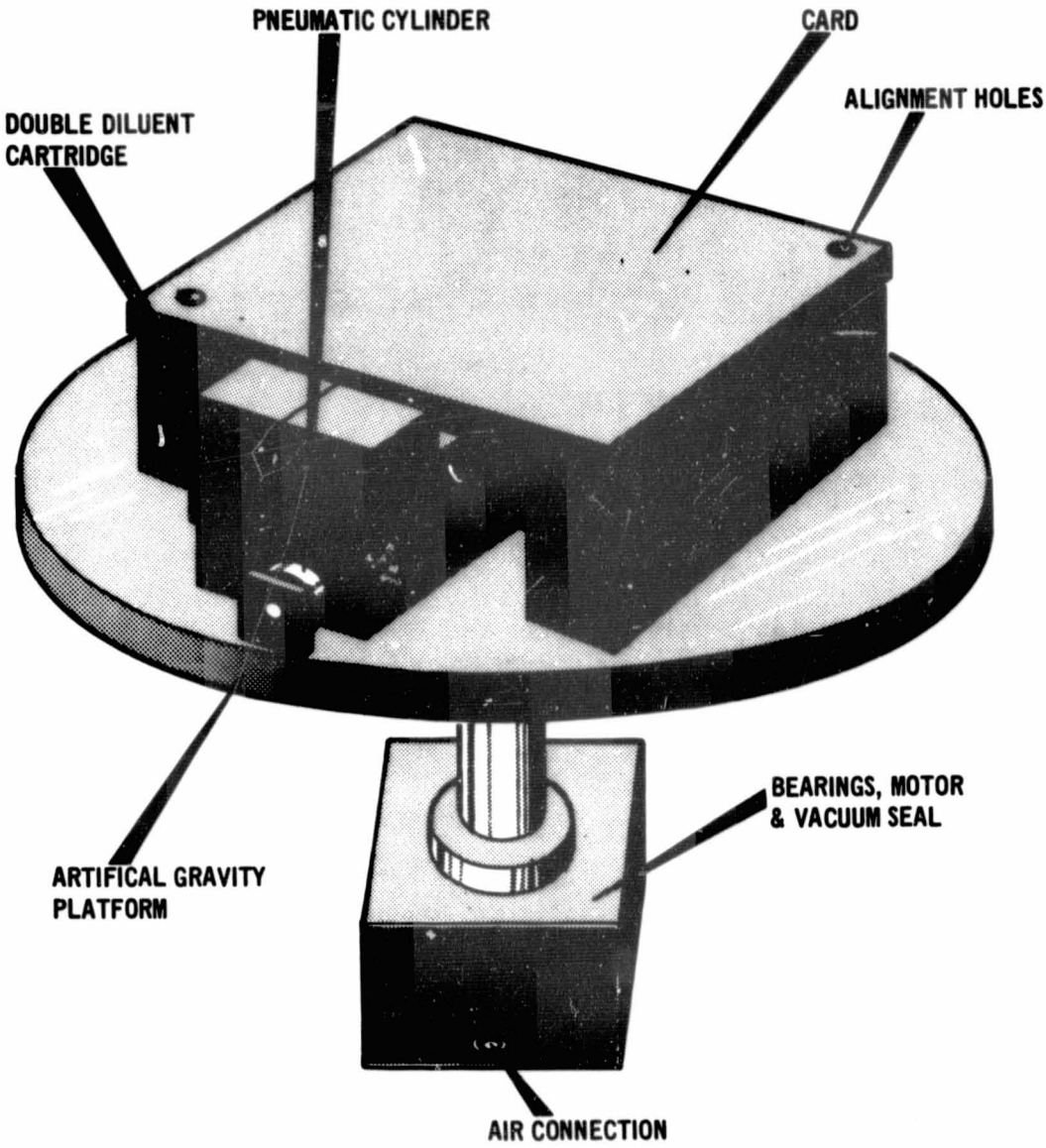

FIGURE 3–28
ZERO GRAVITY FILLING SYSTEM
Concept B

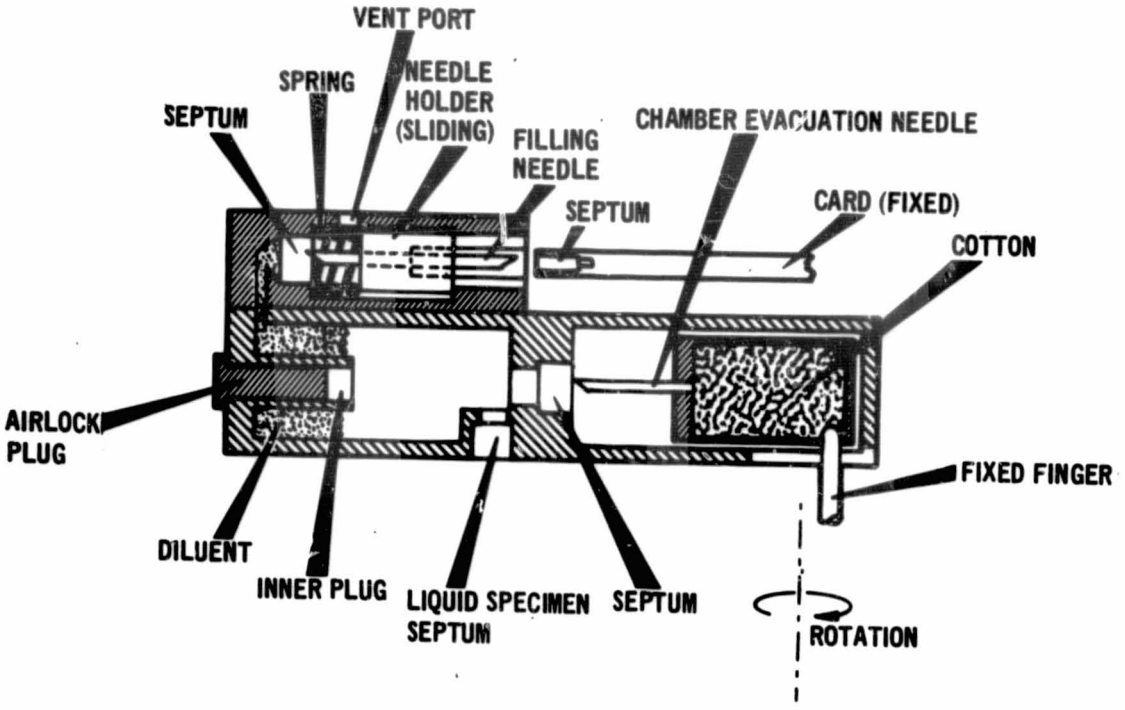

FIGURE 3–29
DILUENT CARTRIDGE
Concept B

The procedure for filling a Card with Concept B starts with the general method of mixing sample and diluent described above. The diluent cartridge is then placed onto the turntable with the fixed finger inserted into the cotton chamber. The Card is placed onto its alignment pins above the cartridge. Both the Card and the cotton filled evacuation chamber remain fixed during the filling process. When the rotation of the turntable reaches a speed sufficient to place all the diluent at the airlock end of the cartridge, a pneumatic cylinder is activated. The pneumatic cylinder advances the diluent cartridge with a force just sufficient for the filling needle to penetrate the Card septum but not to overcome the preloaded spring thereby preventing the filling needle from entering the cartridge septum. The force also causes the cartridge evacuation needle to penetrate the evacuation septum. When vacuum is applied to the SLS, the Card and diluent cartridge are evacuated to the vacuum chamber. The diameter and length of the diluent cartridge evacuation needle limits the rate at which diluent is lost through evaporation.

When evacuation is complete, the pneumatic cylinder exerts the additional force necessary to overcome the preloaded spring and causes the diluent cartridge to advance further. Since the filling needle holder is against the Card, the holder remains stationary. Therefore, the upper diluent cartridge septum is pushed onto the filling needle. With all septums now pierced, the chamber pressure is slowly returned to normal forcing the diluent into the Card. The diluent cartridge is then returned to its initial position by a combination of centrifugal force and release of pneumatic cylinder pressure. Turntable rotation is stopped and the Card can be removed and placed into a reading station.

With its ability to use more than one diluent cartridge, Concept B is a more versatile SLS. Urine enumeration is possible with two cartridges. With three, a simultaneous three sample fill for environmental testing is possible. Concept B requires a larger volume to be evacuated during the filling procedure and also requires one major rotating seal on the vacuum and minor rotating seals for the pneumatics.

3.2.2.4 SLS Concept C Flight Prototype - The cartridge of Concept A was limited by the rotating seals (needles) and the width of the adapter necessary to mate with the Card. Its design has been simplified through the use of a magnetically driven impeller to establish a preferred fluid orientation in a null-g environment, Figure 3-30, Sample Receiving and Card Loading Device. Instead of the whole cartridge rotating, only the impeller rotates and it is driven by an external magnet. This allows the adapter to be incorporated into the device and reduces the spacing required between devices to slightly less than the Card requires for reasonable size. The impeller includes a Teflon covered magnet for coupling to a motor driven magnet external to the vacuum chamber, Figure 3-31. The methods of introducing specimens are similar to the previous designs. A specimen on a cotton tip swab is inserted into the solid specimen port, and the swab handle is broken off and removed. The plunger is then inserted into the port, pressing the swab tip and port plug into the diluent chamber, with the plunger sealing the port. Liquid specimens are injected through the liquid specimen septum. The descriptive name for this device became the "Sample Receiving and Card Loading Device" (SRCLD).

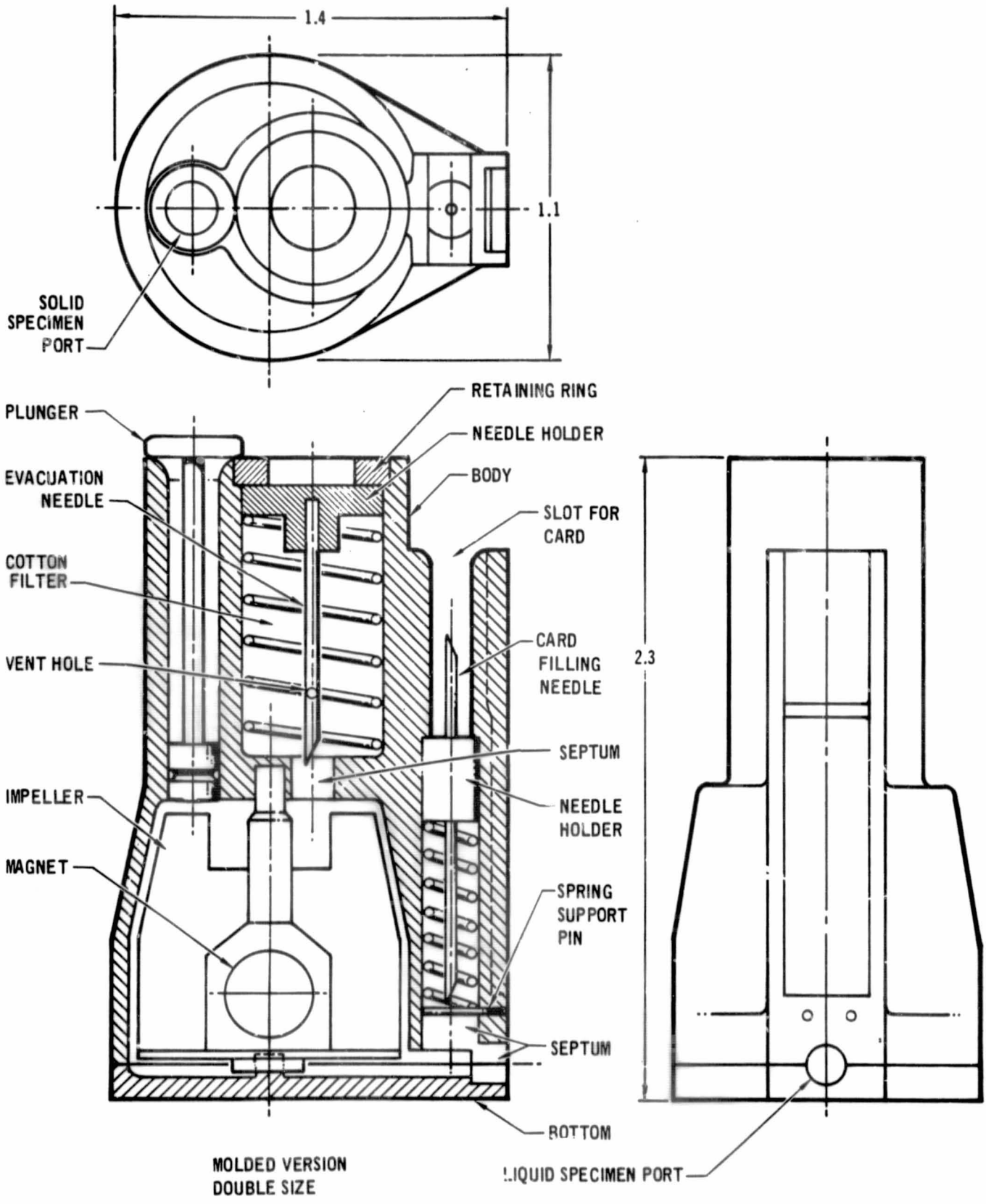

FIGURE 3–30
SAMPLE RECEIVING AND CARD LOADING DEVICE

MICROBIAL LOAD MONITOR

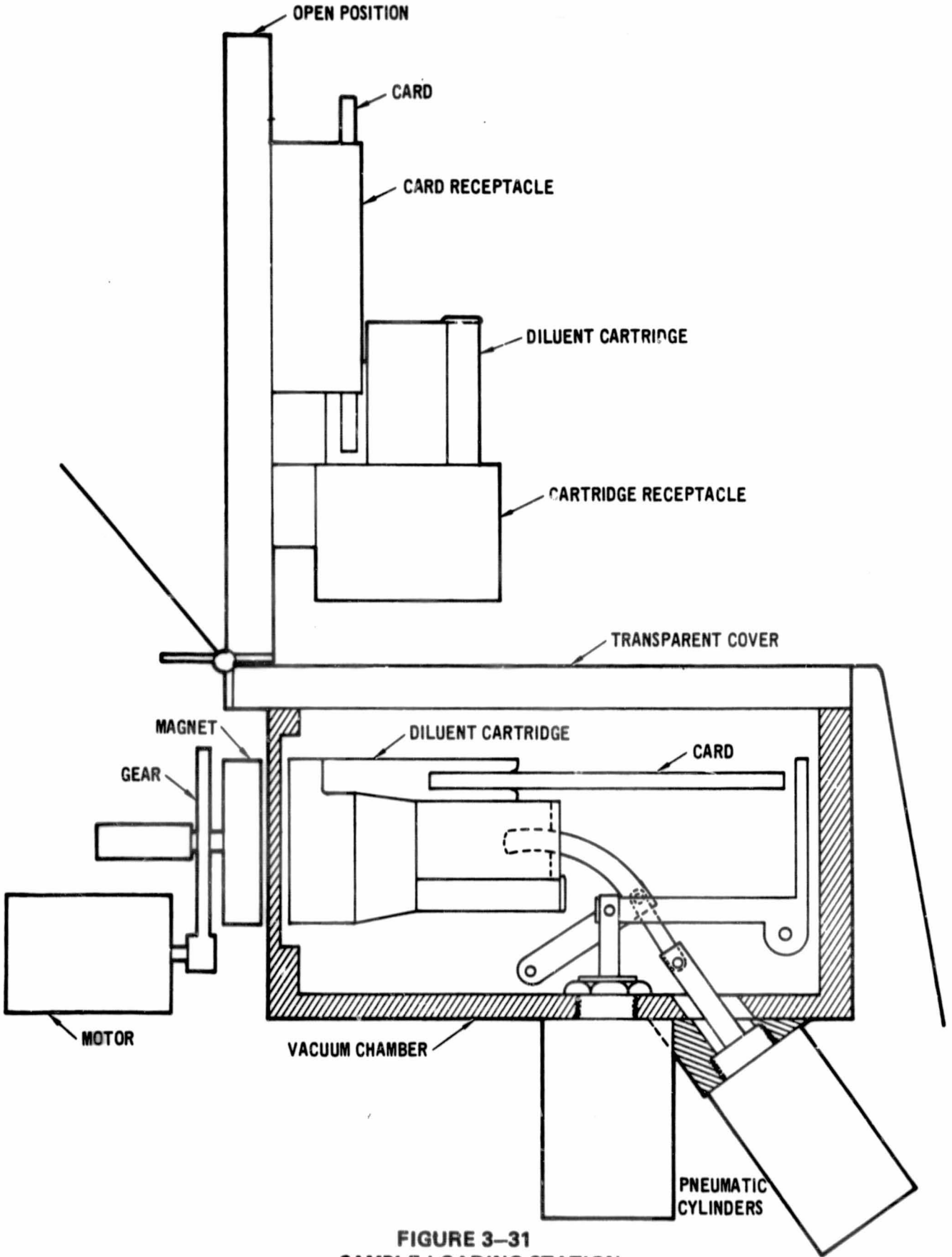

FIGURE 3–31
SAMPLE LOADING STATION

The operation of the Sample Loading System, Figure 3-32, is as follows. The diluent SRCLD or SRCLDs (up to three) are placed in the receptacle of the SLS. The Card is then inserted into the Card holder and onto the SRCLD filling needles. The chamber cover is closed, sealing the vacuum chamber. The magnets next to the vacuum chamber wall are spun to drive the impeller in each SRCLD. In addition to orienting the diluent, the impellers thoroughly mix the diluent and inoculum. An additional advantage to the impeller method versus the rotating cartridge method is the ability to remove small quantities of fluid and introduce it into another SRCLD without air bubbles. This provides a method of dilution under null-g without introducing air bubbles.

Next, a pneumatic cylinder utilizing vacuum instead of pressurized gas presses the evacuation needle into the diluent chamber to evacuate the gases through the cotton biofilter and into the SLS chamber. The Card is evacuated through the double ended filling needle. After approximately five to six minutes of good vacuum (approximately 1 mm hg absolute pressure) a second pneumatic cylinder presses the Card so that the other end of the filling needle enters the diluent chamber. As the chamber pressure is slowly raised, the inoculum backfills into the Card. A pressure transducer and the microprocessor allows monitoring the chamber pressure and switches to fast fill. This dual mode allows less pressure differential across the tape interface of the wells and, therefore, less tape distortion. Time is allowed at ambient pressure for the Card pressure to stabilize and then the pneumatic cylinders are deactivated, with springs retracting the needles from the diluent chamber to prevent leaks. The Card is then removed from the Sample Loading System and placed in a reading station. The SRCLDs should be disposed of properly as regards contaminated material.

The SLS Evacuation System schematic is shown in Figure 3-33. The SLS is designed to require only vacuum and a small quantity of electrical power. The vacuum actuators operate on the pressure differential between the vacuum source and the cabin atmosphere which can be as low as 10 psig. The sequencing of the vacuum actuators has been planned to avoid compromising the chamber vacuum during the critical portion of the loading cycle. The chamber evacuation valve is a vacuum operated vacuum valve. There are four miniature solenoid valves to control the operation of the SLS. The first solenoid valve is the pilot valve for the chamber evacuation valve. The second solenoid valve permits the fast bleeding of the

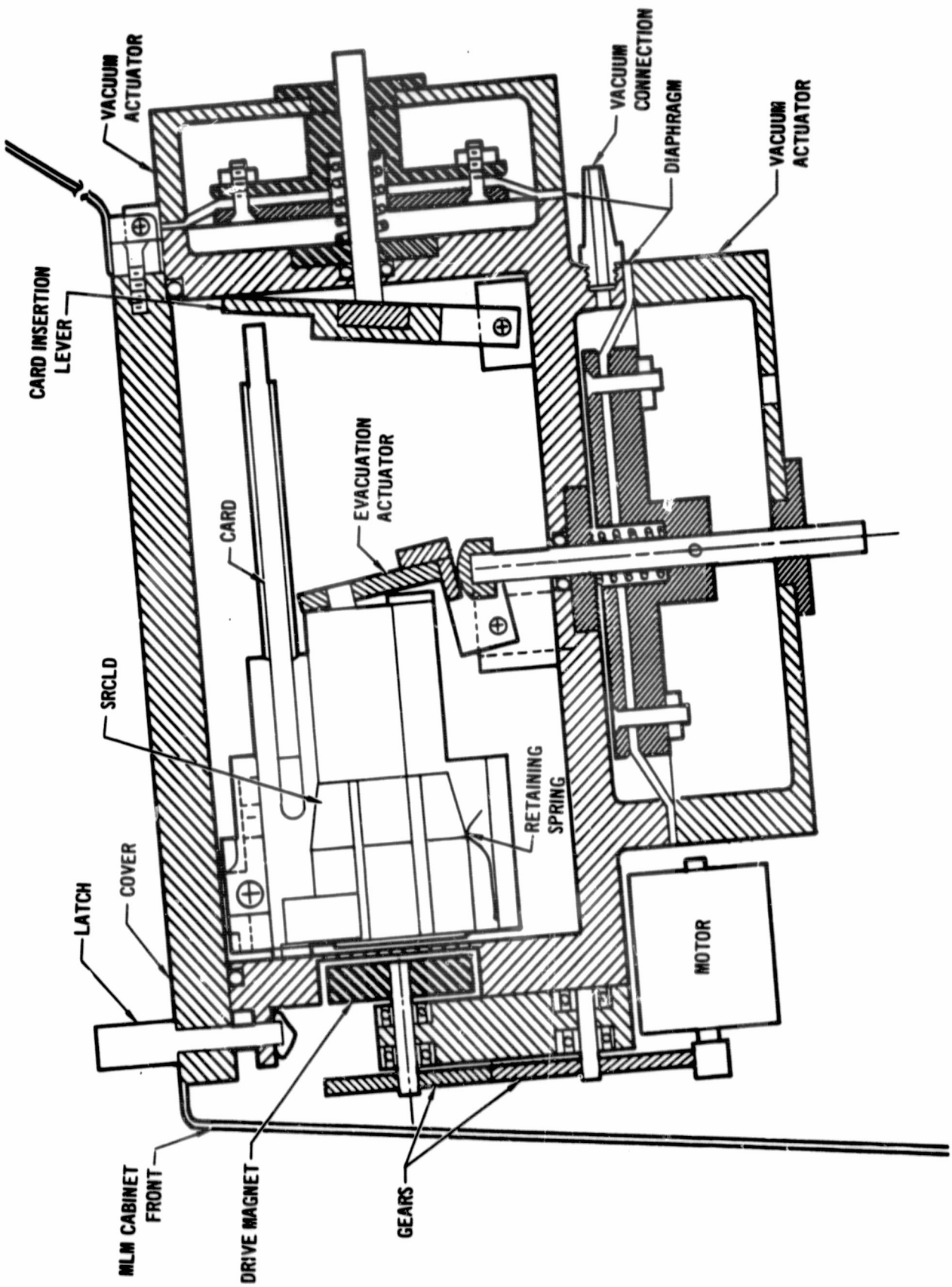

FIGURE 3–32
SAMPLE LOADING SYSTEM

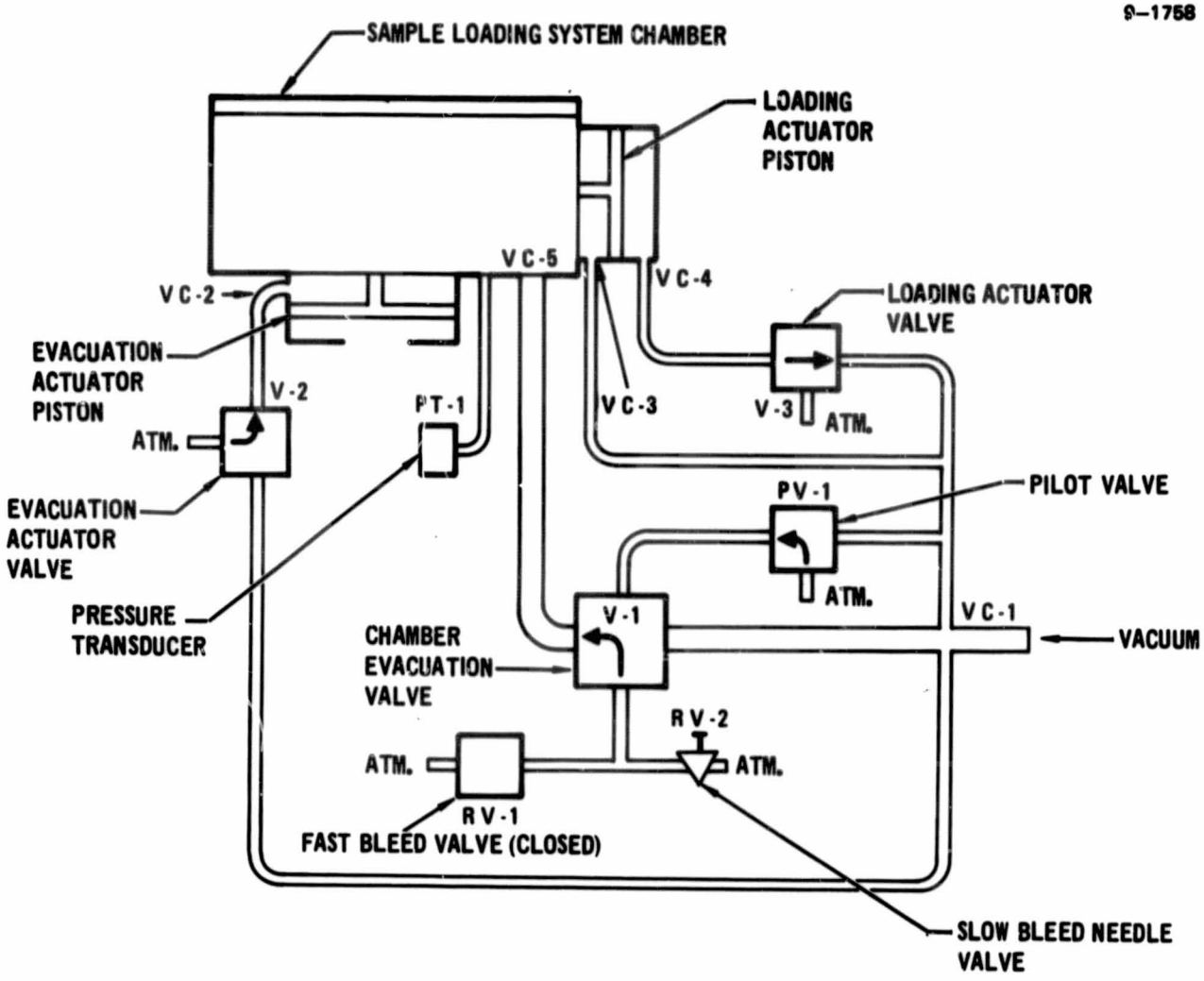

FIGURE 3–33
SLS EVACUATION AND ACTUATION SYSTEM
(Valves Shown in Deactivated State)

chamber back to atmospheric pressure. Without it, returning the chamber to atmospheric pressure would take several minutes. The other two solenoid valves control the vacuum actuators. Each solenoid valve requires only 0.65 watts of power.

3.2.3 MLM Incubation and Detection Instrument - The criteria for MLM instrumentation developed at the outset of the program stressed simplicity, reliability, and high density. The first generation equipment designed to work with the air impaction sampling system utilized a dual arrangement of solid state light emitting diodes (LED) and silicon detector diode pairs, and all solid state electronics to achieve reliability.

An analog circuit configuration was selected for simplicity using proven electronic components for the feasibility evaluation hardware. Several analog MLM instruments were fabricated with six channels (three dual emitter/detector pairs and the associated electronics). These were used extensively during Phase II (demonstration of the MLM system diagnostic capability). The term "channel" is used when describing the electronic portion of the MLM and the term "well" is used for the biological portion mainly associated with the cassette or Card. During the last quarter of Phase II, a four channel digital MLM instrumentation system was designed and fabricated. This design eliminated several objectional features of the analog instruments including (a) large drift in output data, (b) complex set up procedure with interacting controls, and (c) limited functional range of media density. The four channel unit was expanded to accommodate the five channel filter cassette and this combination served as the baseline for the second generation MLM instrumentation development.

The development of the Microbial Load Monitor instrument has closely paralleled the development of integrated circuits. Initially transistors and analog integrated circuits were used to detect organism growth. High analog gain was necessary due to the relatively weak LEDs and photodetectors then available. Then came digital electronics and multiplexing of the preamplified detected signal. This allowed electro-optical digital readouts, digital data printer, and direct calculator processed information. Electronic drift was greatly reduced and most of what remained was the same from one channel to the next.

The four channel digital evaluation model concept is shown in Figure 3-34. Each channel consists of the electronics between input multiplexor (emitter side) and the output multiplexor (detector side). Whereas the analog system had used dedicated electronics for each emitter/detector pair (electrooptical channel), the digital concept multiplexes through the channels turning on each LED sequentially while simultaneously selecting the corresponding preamplifier output for processing. Several benefits evolve from this approach, the most significant being the electrical power saving resulting from greatly reduced LED average current. For example, there is approximately five watts difference saved in a ten channel system. Second, the reduction in electronic components saves weight, size and increased reliability by simplifying electronic circuit boards and interconnecting wiring.

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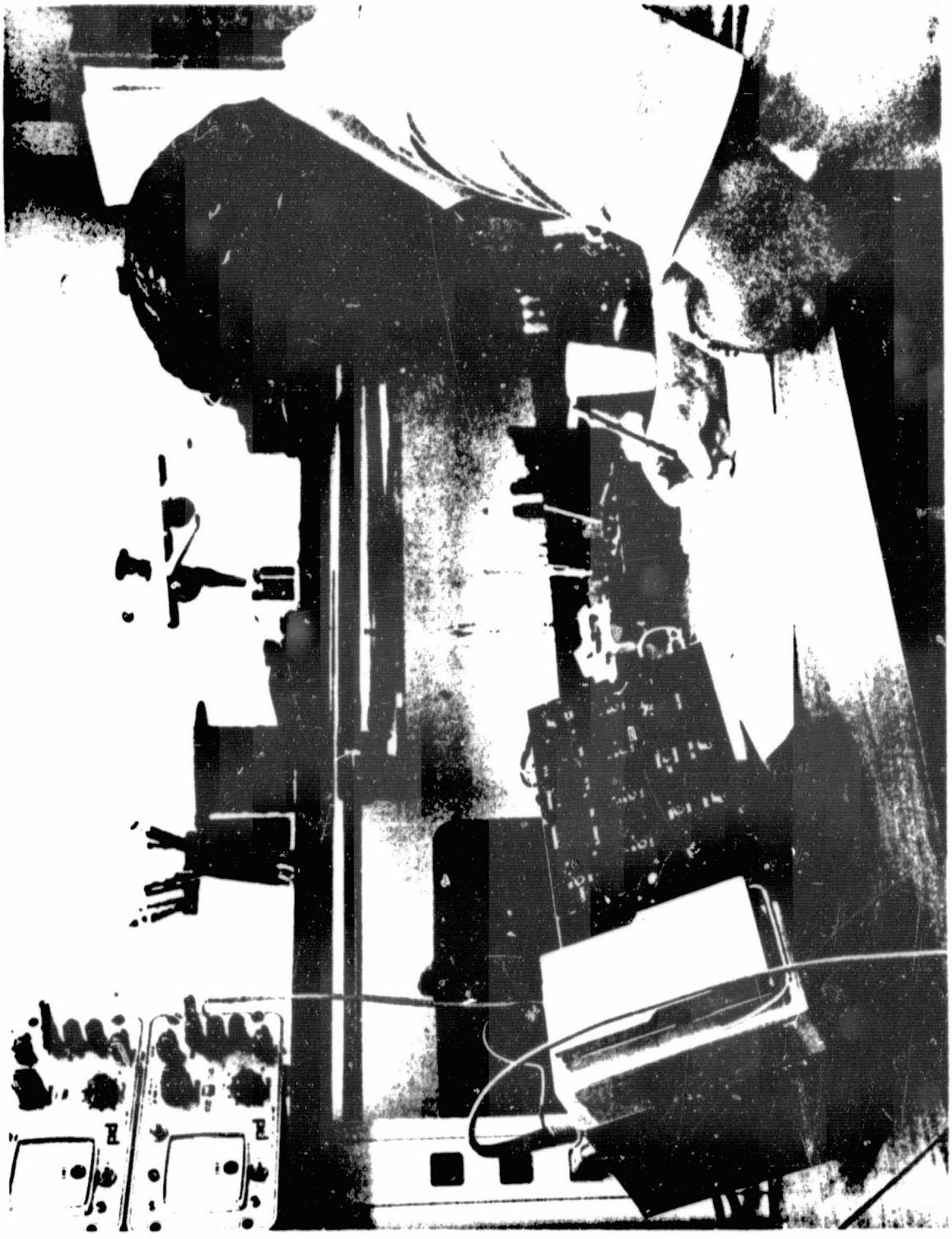


FIGURE 3-34
DIGITAL EVALUATION MODEL

The four channel laboratory evaluation model was used to evaluate the digital sequential sampling concept. Subsequently, the unit was increased to a five channel capacity to accommodate expanded microbial test requirements or four dilution stages and one control. This five channel unit was used extensively to evaluate the basic system stability, and to evaluate new media and antibiotic sensitivity formulations.

The next requirement was for the construction of a semi-automated sequential sampling (digital) laboratory test model. A block diagram of the concept is shown in Figure 3-35. Implementation of this system included a multichannel optical

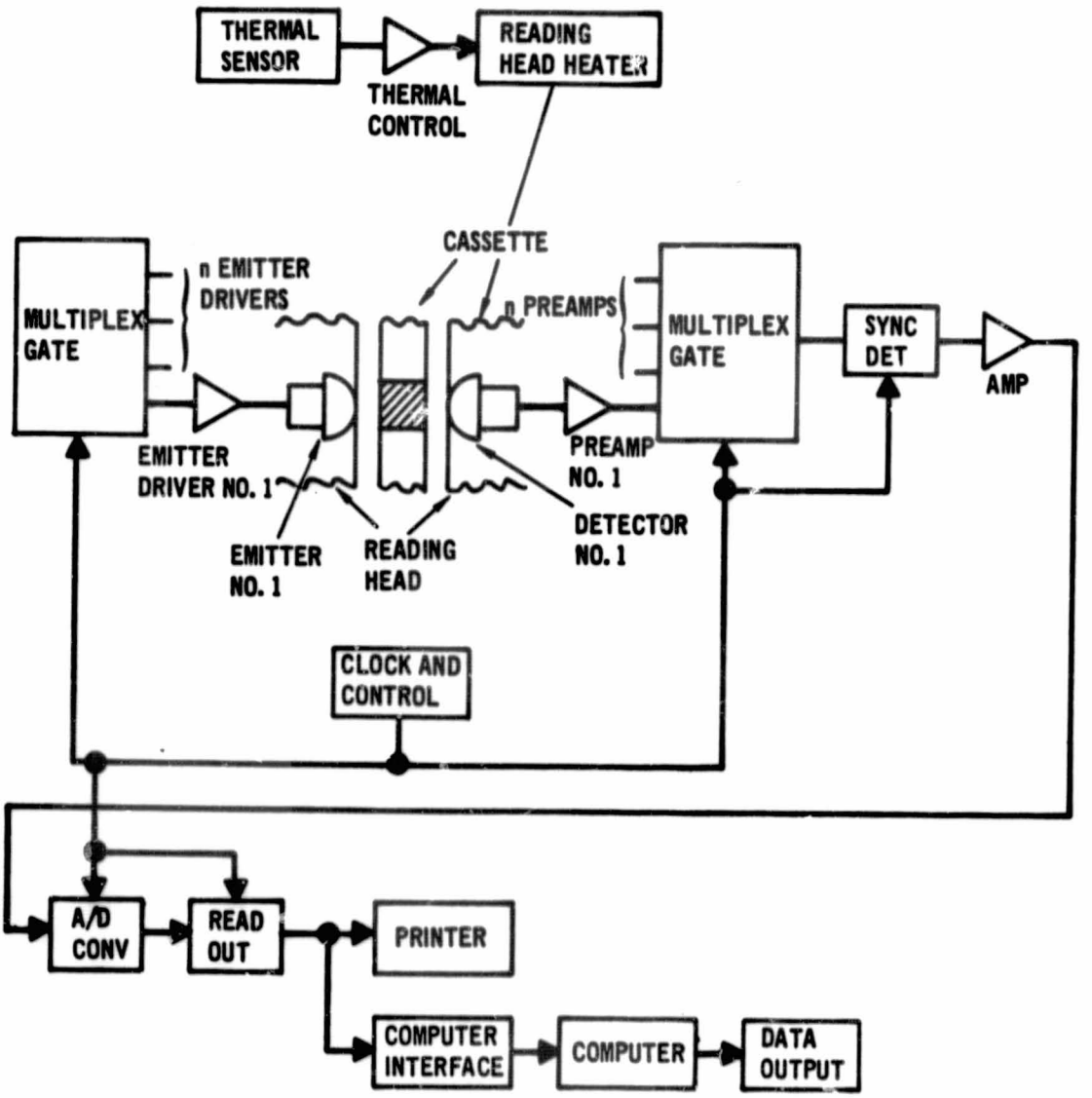


FIGURE 3–35
SIMPLIFIED DIGITAL MLM ELECTROOPTICAL DETECTION SYSTEM

densitometer with digital signal processing and computer processed microbial growth time history data. Such an implementation scheme is shown in Figure 3-36.

The MLM instrumentation was designed to accommodate up to 100 optical channels (10 ten-channel cassettes). The breadboard configuration shown in Figure 3-37 includes five reading stations, each with the capability of either two antibiotic cassettes, two dual cassettes described previously, or a combination. The electronic circuitry is contained behind and below the control panel. The circuitry consists of 34 printed circuit boards, interconnecting cable harness and various electronic components. Beneath the reading heads for each 10 channels there are two five-channel hybrid thin film integrated preamplifier circuits mounted on both sides of a printed circuit board. Each channel is individually calibrated with a potentiometer accessible from the back panel. Functionally, this instrumentation circuitry is identical to that of the five-channel model consisting of an electrooptical section, a clock and control, a digitizing section, and a display and data output.

3.2.3.1 High Volume Instrument - Previously the design of the MLM system has been directed toward developing a diagnostic capability with a few samples per day from a small population in a space environment. The objective of Amendment 1C to MLM Contract NAS 9-11877 was to turn around the hardware development, and to provide high volume cassette handling capability. The Statement of Work established an increase in handling capacity from 10 cassettes for the space application (MLM-S), to 150 cassettes for a high volume instrument for earth application (MLM-EA).

The major design change, resulting from the large increase in MLM cassette processing capacity, was in the cassette handling system. To meet the new requirements at reasonable cost, the cassette handling was changed from a static (remaining in the reading head at all times) system to a dynamic cassette handling system. The goal of processing 150 (ten-channel) cassettes was instrumental in the design evolution. A major feature of this design was a carrousel for handling the 150 cassettes. By designing the cassette handling system to recycle each cassette every thirty minutes, it is possible to obtain 30 minute time history profile data on up to 150 cassettes.

FIGURE 3–36
SEMI-AUTOMATIC LABORATORY TEST SCHEME

FIGURE 3–37
MLM-S BREADBOARD

3.2.3.1.1 High Volume Carousel - The major unique elements in the high volume carousel design included: (a) cassette transport assembly, (b) carousel sequencing mechanism, (c) transfer mechanism, and (d) reading station assembly.

In the initial concept of integrating the high volume carrousel assembly with the existing 100 channel MLM-S electronic instrumentation, 50 channels of MLM-S capability was left unfinished and used for interfacing to the carrousel assembly. As shown by the Figure 3-38 diagram, 50 channels were planned for the growth data acquisition. With this five head scheme the carrousel rotation rate was 2.5 hours per revolution. Readings taken every 30 minutes from each of the five heads had to be reconstructed by the data acquisition system for processing and presentation. The system was integrated with the MLM-S flight breadboard electronics and coupled to the Wang data acquisition system as depicted by the Figure 3-39 pictorial.

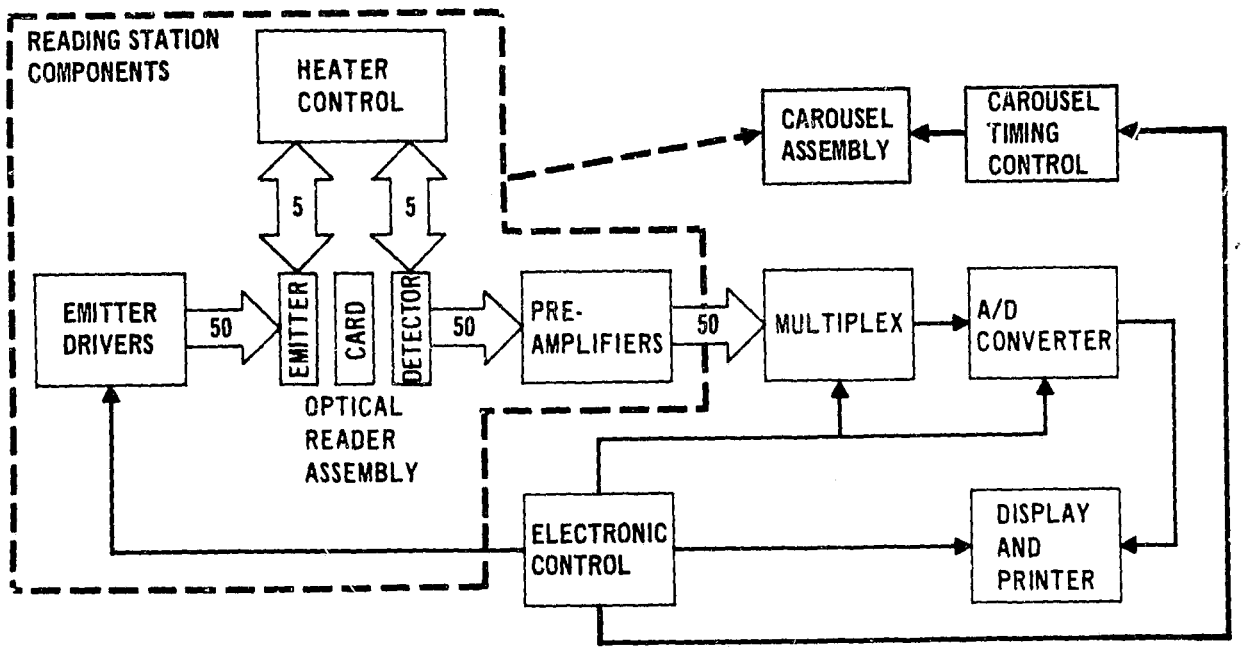


FIGURE 3–38
50 CHANNEL MLM – EA

The cassette transport assembly performed the dual functions of retaining the cassettes in a controlled thermal environment and of advancing them sequentially to the optical reading station. Figure 3-40 shows the 150 cassette carousel transport assembly. Four central inlet holes (around the pivot point) coupled with 150

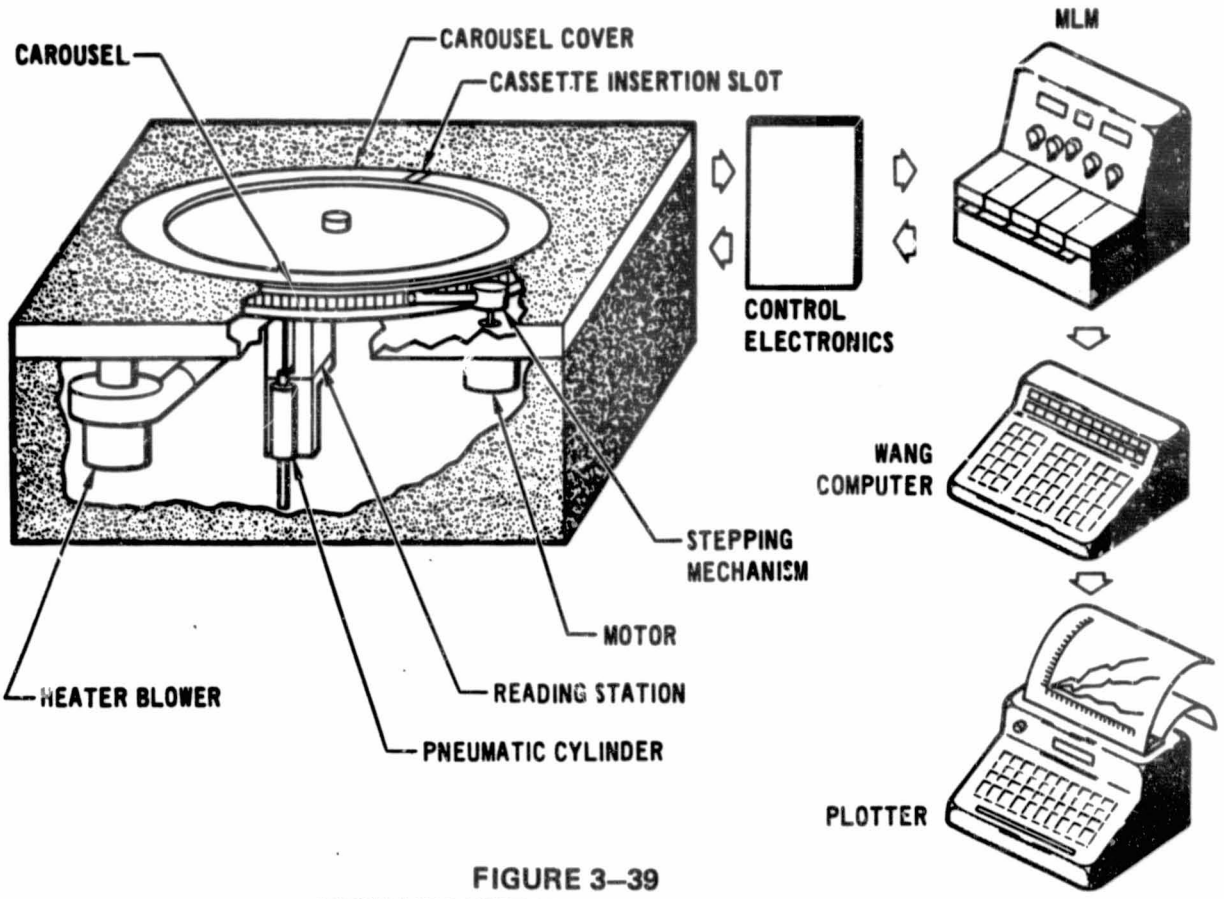


FIGURE 3–39
HIGH VOLUME MLM BREADBOARD

cassette related exhaust slots allow continuous circulation of temperature stabilized air to flow around the cassettes during incubation. Each slot is configured such that the cassette can be easily extracted through the bottom of the assembly for insertion into the reading station.

The sequencing mechanism is shown in detail in Figure 3-41. A 25 rpm motor drives the stepping key through an eccentric cam. The key pushes on the partition separating the cassettes in the carousel. The inherently accurate stepping mechanism is easily adjustable to compensate for wear. The sequencing is initiated by a stepping pulse from an electronic controller. A microswitch (not shown) contacting the rear of the cam block is adjusted to allow one cassette advance of the carousel and to properly position the stepping key at the end of travel.

A detailed view of the cassette transfer mechanism and reading station is diagrammed in Figure 3-42. In proper synchronization with the transport assembly, the pneumatic cylinder raises and lowers a finger aligned with a notch in the

9–1752

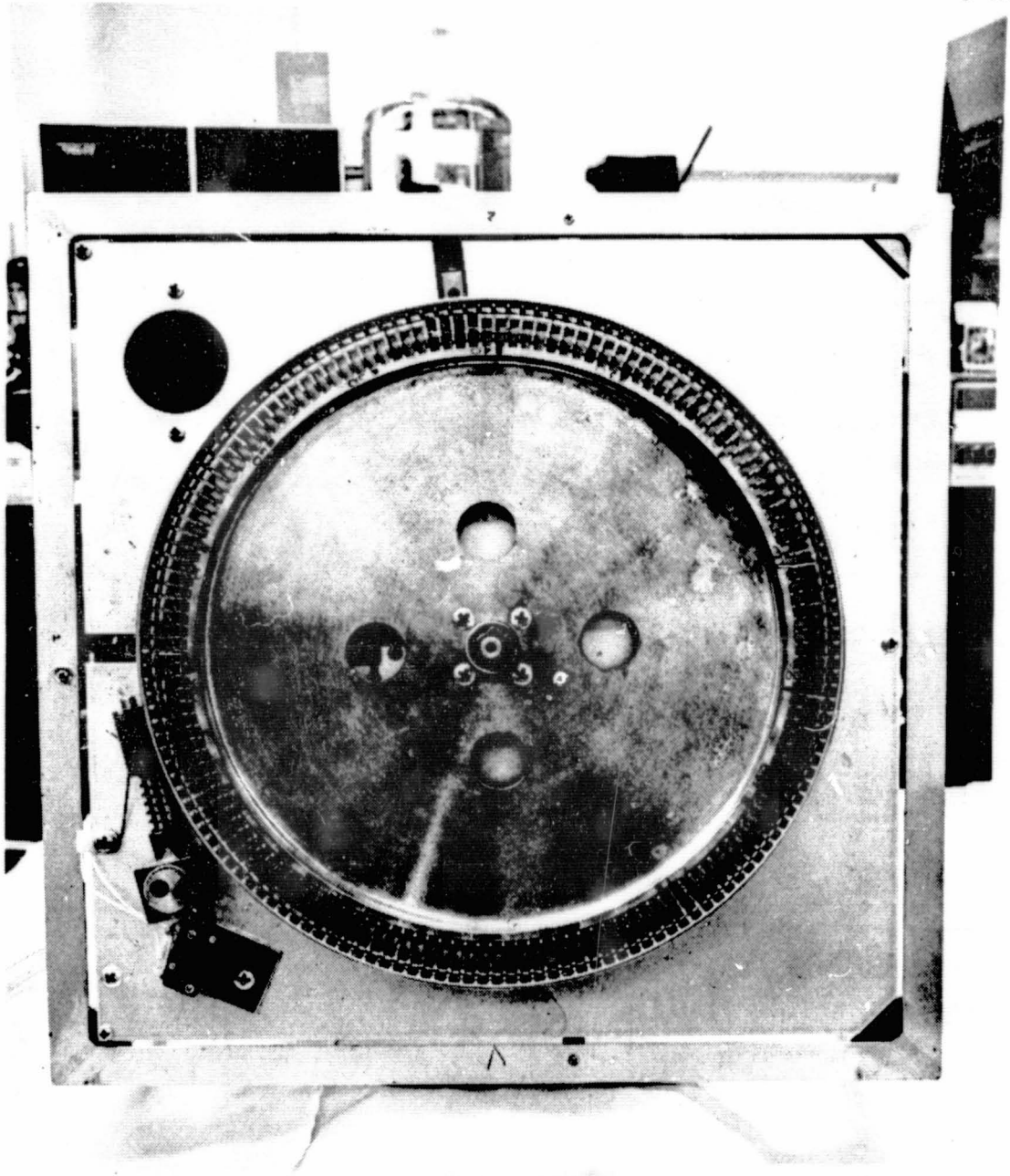


FIGURE 3–40
150 CASSETTE TRANSPORT ASSEMBLY

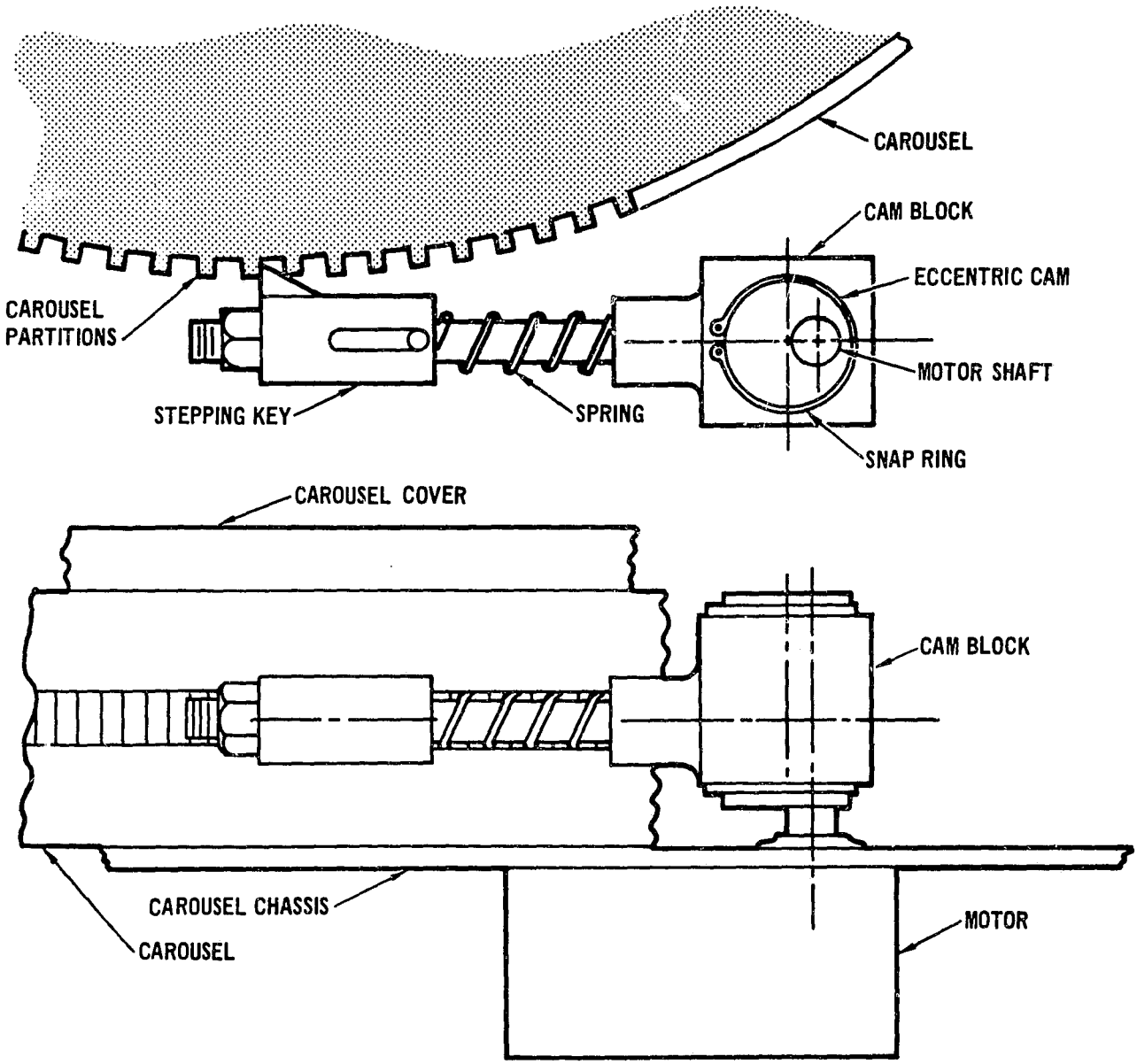


FIGURE 3–41
CAROUSEL SEQUENCING MECHANISM

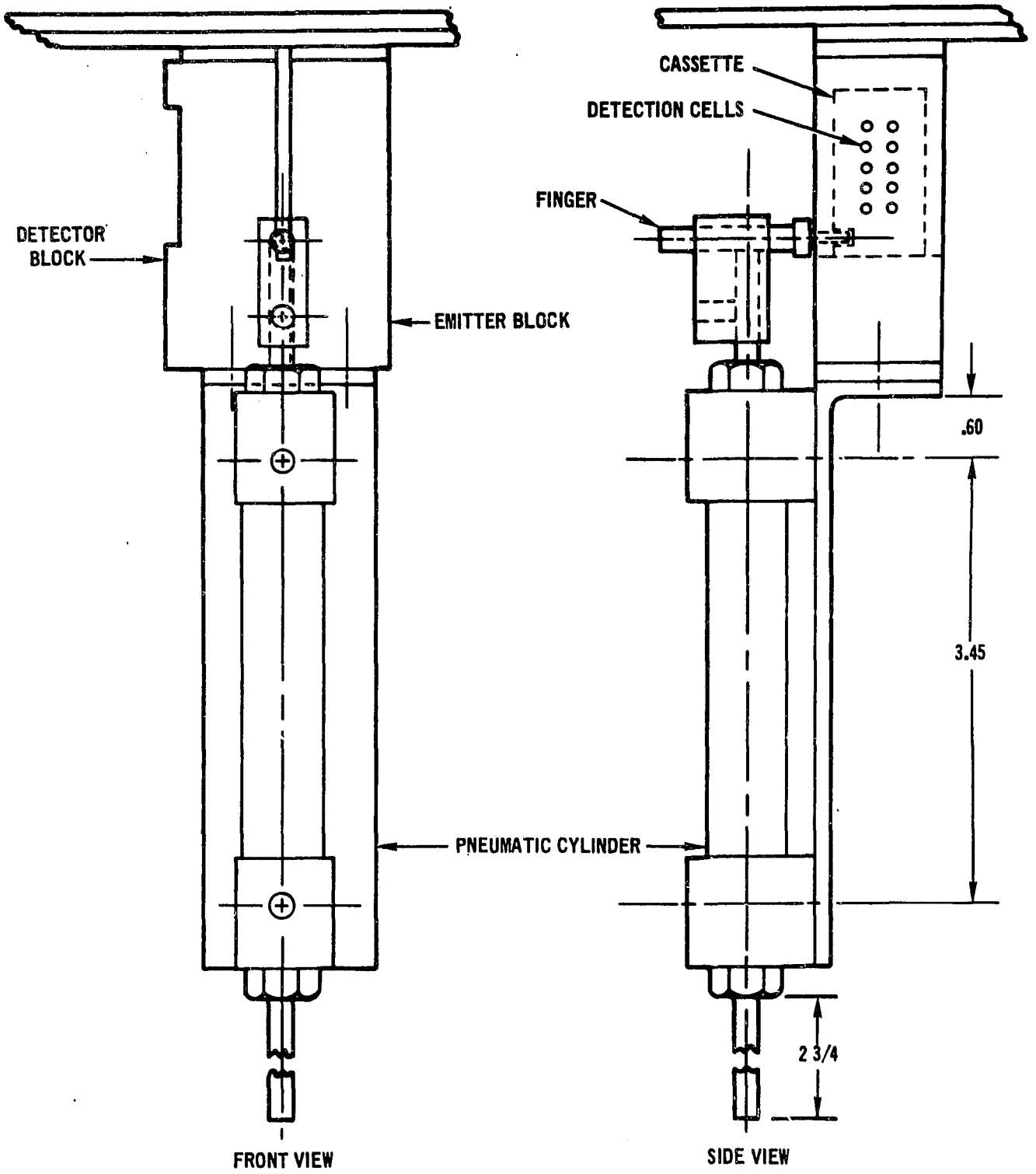


FIGURE 3–42
CASSETTE TRANSFER MECHANISM AND READING STATION

cassette. Springs guide the cassette into the reading station and hold it against the detector block. Precise timing of the cassette sequencing transfer and data acquisition assures proper positioning of the cassette against the reading head guides before optical density readings are taken.

The carousel transport, sequencing mechanism, transfer mechanism and reading head assembly are visible in the Figure 3-43 photograph. Top and front metal covers are removed in this view. The sequencing mechanism is seen at the right-front portion of the top plate. The transfer mechanism and reading head assembly are partially visible behind the vertical structure. A blower recirculates air through the carousel to maintain the cassettes at approximately 36°C. The carousel port assembly is covered with a clear polycarbonate disc to provide thermal stability. The photograph also shows the carousel assembly with the disc in place and demonstrates the technique for loading a single cassette into the transport assembly through the entrance slot in the plastic cover.

Extensive tests with the full up system (five reading stations positioned at 30 minute interval points) provided data values with up to 20% error following normalization of the light intensity variance. These errors were determined to be due to misalignment of the various emitter detector pairs and slight variations in the chip/cover-tape optical characteristics. The minimum impact correction for the problem was a design modification which reduced the number of reading stations to one, increased the carousel recycle rate to 30 minutes/revolution and eliminated the large number of computations required to normalize and reconstruct the data from five reading heads.

3.2.3.2 MLM System Flight Prototype - The MLM system flight prototype is a modification of the breadboard static MLM with its clamshell incubation heads. Figure 3-44 is a photograph of the full instrument. It is designed to be a unitized instrument with both sample loading and detection capability. The Sample Loading System has a capacity to load one integrated Clinical Card in a null-g environment. The integrated Card performs both screening and antibiotic tests for simultaneous detection and susceptibility capability. With the advent of microprocessors that fit entirely on one printed wiring board, the solid optical detection system, operating at 665 nm wavelength, is directly controlled for maximum reliability, flexibility and ease of maintenance. The completed system is shown in Figure 3-45.

9-1817

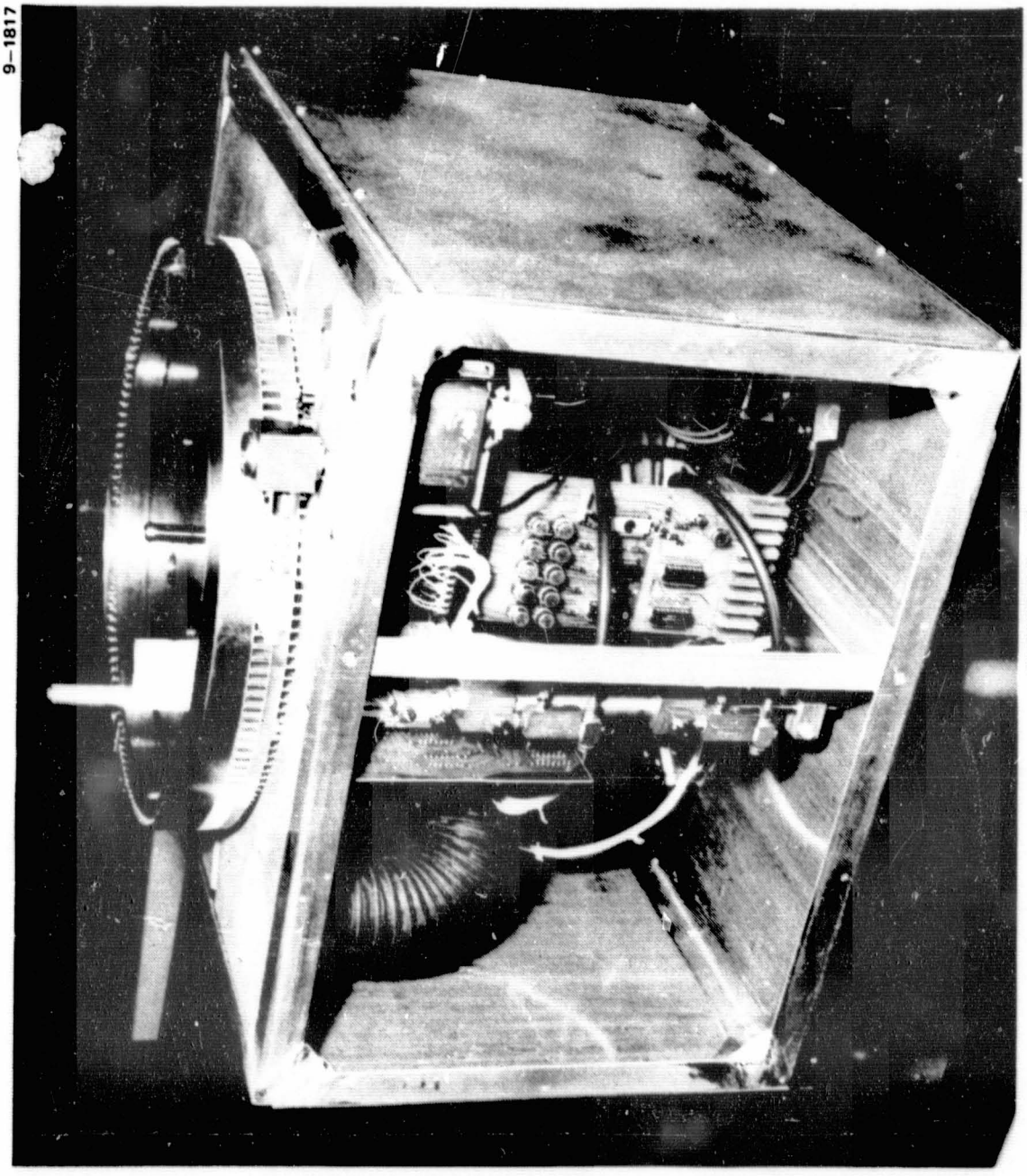


FIGURE 3-43
HIGH VOLUME MLM CAROUSEL ASSEMBLY – INSIDE VIEW

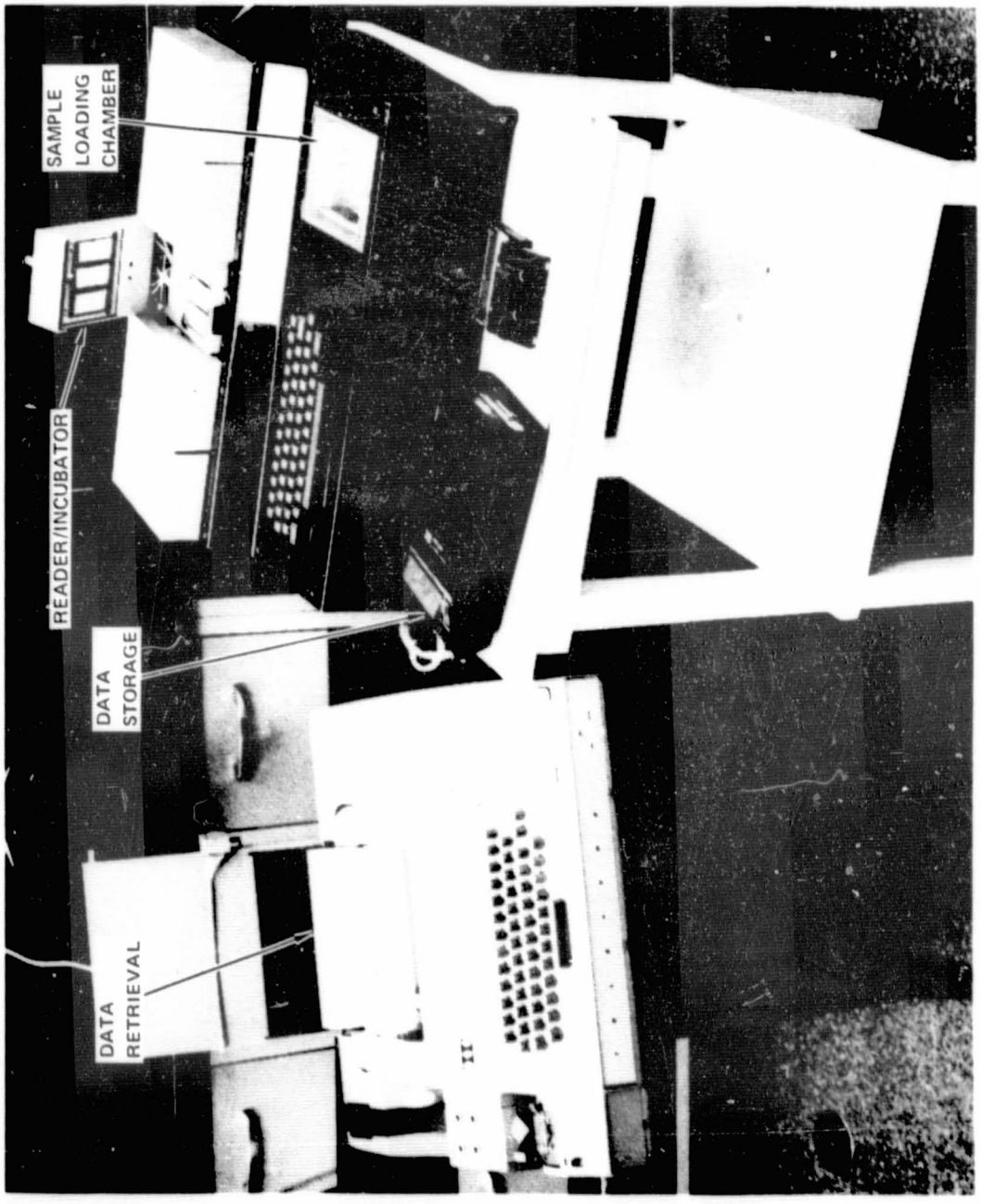


FIGURE 3-44
MICROBIAL LOAD MONITOR
FLIGHT PROTOTYPE INSTRUMENT

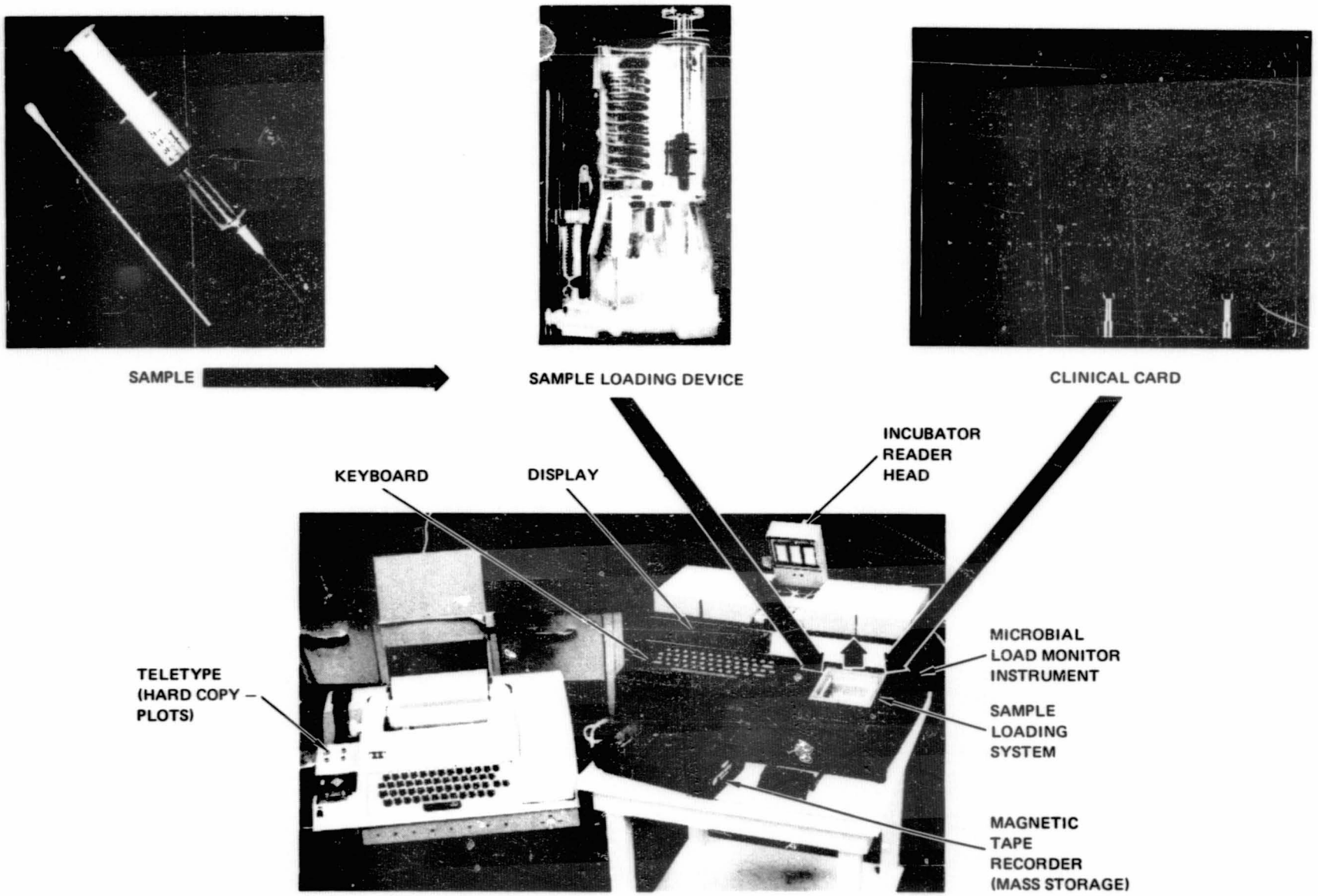


FIGURE 3–45
MICROBIAL LOAD MONITOR SYSTEM

The present Microbial Load Monitor instrument is a complete system including microprocessor, memory (both read/write and PROM), reader/incubator heads, electronic interfaces and peripherals, power supplies, and Sample Loading System. The following describes these areas and the design philosophy. The Sample Loading System (SLS), an important part of the present MLM, was described in Section 3.2.2. The basic philosophy was simplicity, completeness, and no major design problems in converting to a unit for use on board a spacecraft.

3.2.3.2.1 Microprocessor - The microprocessor selected in 1974 to control the MLM flight design is an IMP-16/C National Semiconductor Corporation 16 bit parallel processor. The IMP-16C at that time was the only available single board microprocessor available with a related software development system. The IMP-16C printed wiring board is shown in Figure 3-46. This printed wiring board (8-1/2 x 11 inches) has 256 words of semiconductor read/write memory (unused) and sockets for 512 words of semiconductor read-only memory (ROM or PROM). Additional memory is held on additional boards similar to the lower boards. The terms microprocessor and microcomputer are nearly synonymous with microcomputer having the characteristics and use of a more general purpose device rather than dedicated.

The IMP-16C includes a 16 bit address bus, a 16 bit input bus, a 16 bit output bus and various control signals such as memory and peripheral data input and output flags. Other general purpose flags and sense lines are available to the hardware designer and programmer, many of which are used in the MLM. Through these buses, flags, and sense lines the microprocessor controls and calibrates the MLM emitter/detector pairs in the incubator heads, sets and reads the real time clock, performs arithmetic processing on the detected signal and compares threshold values and data values for growth/no growth decisions, and communicates with the operator of the MLM. Calibration of the many detector pairs is accomplished accurately and rapidly by controlled use of the D/A and A/D converters.

3.2.3.2.2 MLM Memory - The original design included a memory board and controller from National Semiconductor Corporation. These boards are shown in Figure 3-46 with the memory board on the left and the controller board on the right. Use of these boards on a spacecraft was questioned since they require considerable power and would be difficult to back up with batteries. A redesign was done using CMOS memory devices. These are extremely low power semiconductor devices both in operation and in standby use. Two boards were fabricated and

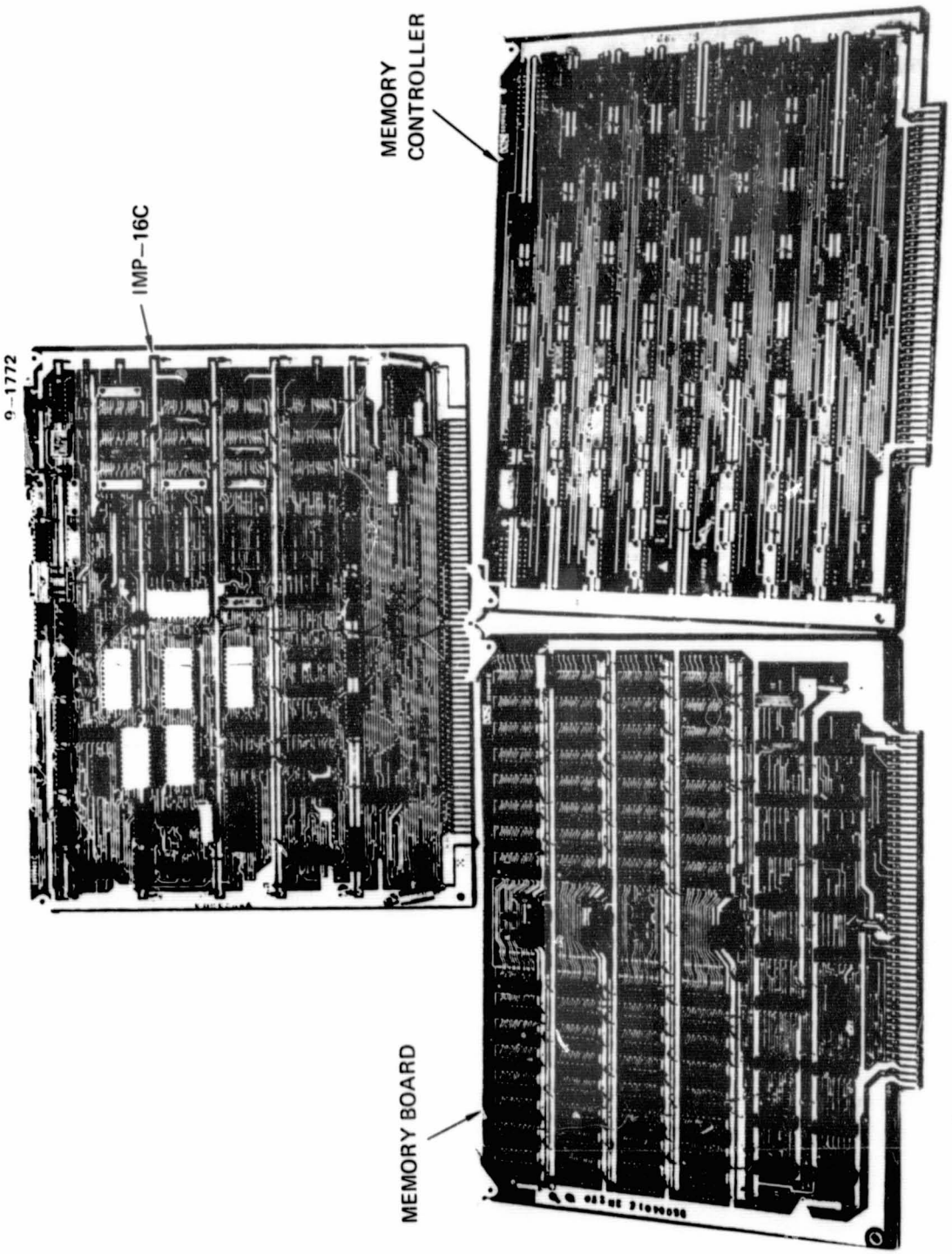


FIGURE 3–46
IMP-16C MICROPROCESSOR AND MEMORY BOARDS

operated for a period of time during software development in the MLM. One CMOS memory board has subsequently been replaced with an 8K by 16 bit EPROM board from National Semiconductor Corporation. This allows all programs to reside in memory at the same time.

After reviewing three devices on the basis of cost, complexity, and power consumption, the Intel P5101L 1K memory chip was chosen. While the P5101L is not the optimum device with regard to cost and circuit complexity, it has the advantage of extremely low operating and standby power consumption. Since our long range goal was to develop a unit for space use, low power was given the highest priority. The device requires only a single +5 volt supply and is TTL compatible. Maximum power supply drains are 27 milliamps operating current and 15 microamps standby current.

The P5101L offers several options for chip enable functions. There are two inputs for chip enable: CE1 and CE2, and one for output disable, OD. CE2, however, will fully enable or fully disable the entire device (assuming CE1 is held high and OD is held low). To minimize the MLM memory complexity, CE1 and OD are tied to their appropriate logic levels and only CE2 is used to operate individual chips. Whenever CE2 is low, the P5101L is completely disabled, independent of all other inputs, and draws only the ultra-low data retention current (15 microamperes maximum).

By using only CE2, read and write cycles are also simplified. A read cycle is distinguished from a write cycle by the condition of the read/write (R/W) input. Reading is accomplished by applying the proper address and bringing the CE2 and R/W lines high. Data is valid at the outputs after 650 nanoseconds. Writing is accomplished by applying an address and the data to be written, pulling CE2 high, and holding the R/W line low. A write cycle is also 650 nanoseconds in length (maximum).

The memory design is, therefore, fairly general except for some added features required, either to interface the P5101L with the IMP-16C system, or to allow for power-fail data retention. The CMOS memory system schematic is shown in Figure 3-47. Because of timing differences between the IMP-16C and the P5101L memory cycles, data latches were needed on the data inputs to assure valid data throughout a write cycle. The 7400 and 74LS00 series TTL devices were required as peripheral logic (address decoders, buffers, and latches) because the IMP-16C, being a MOS/LSI design operates with a memory cycle too short for a totally CMOS design. Rather than modify the IMP-16C memory timing, it was decided to adjust the CMOS memory structure.

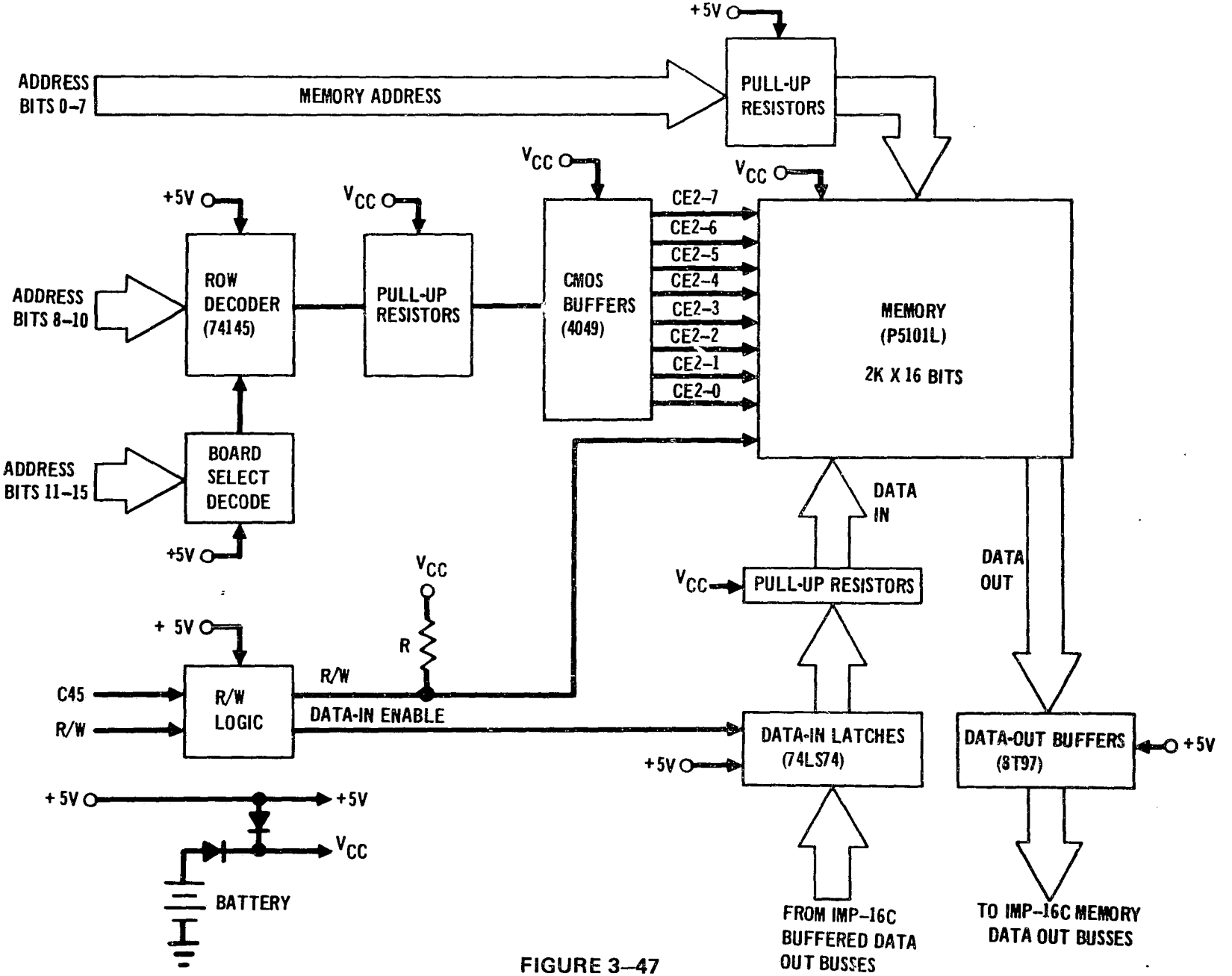


FIGURE 3–47
CMOS MEMORY SYSTEM

Power fail battery backup was accomplished in spite of the TTL circuits present, by splitting the power supply between the TTL circuits and the CMOS devices. When power fail of the main +5V supply occurs, diode switching allows only the CMOS circuits to remain active via the onboard batteries. A novel combination of row decoder (74145), pull-up resistors, and CMOS buffers (4049) allows all chip enable (CE2) lines to be held low when power fails, keeping the memory in the low power state. Batteries chosen for the battery backup are two Mallory No. 10L124 silver oxide batteries, 1.5 volts each. These batteries have a rating of 120 MA hours at a current drain of 1.6 milliamperes. The completed CMOS memory board is shown in Figure 3-48.

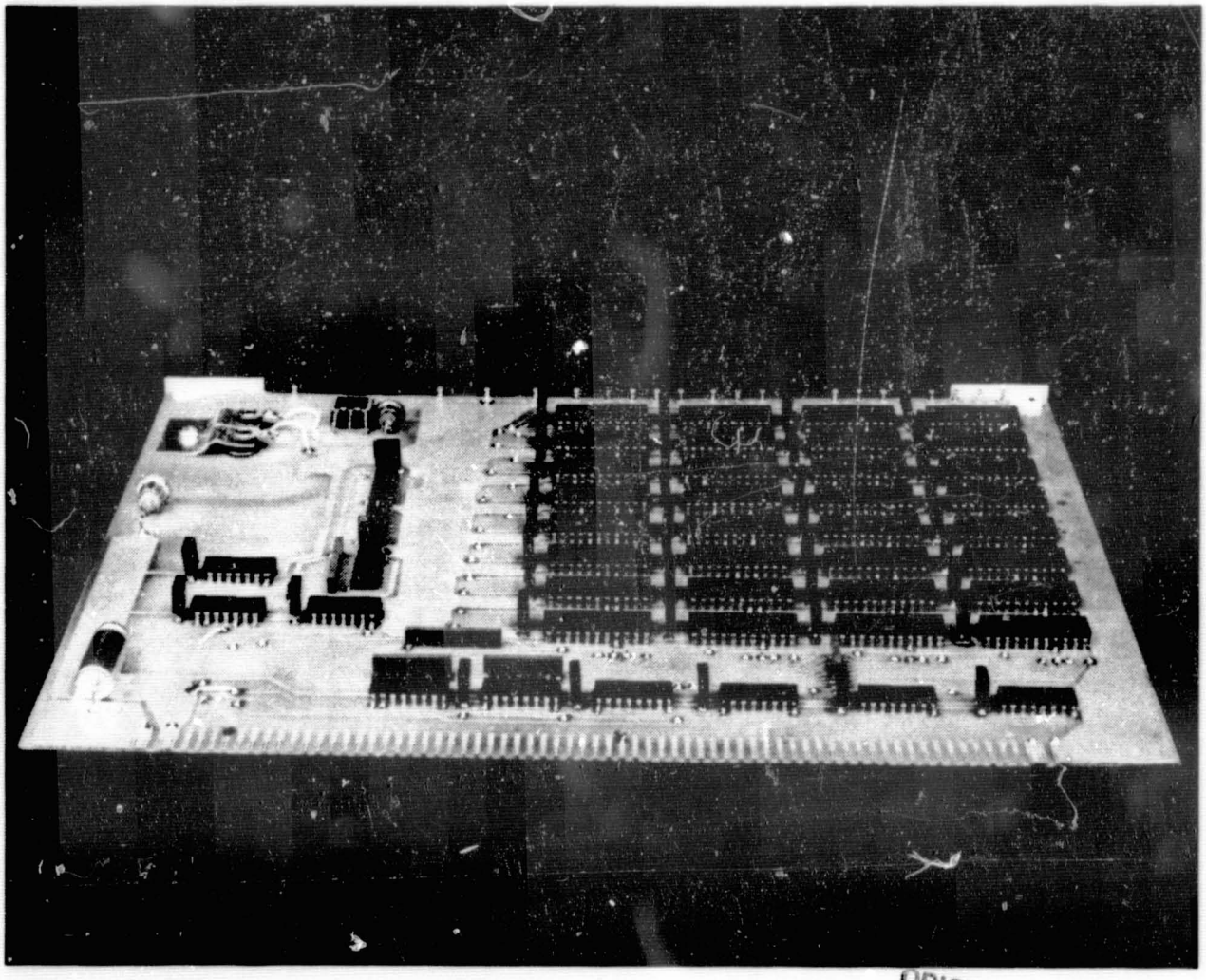


FIGURE 3–48
CMOS MEMORY CARD

3.2.3.2.3 MLM Reader/Incubator Head - The design of the reader/incubator heads includes the head electronics, the incubator design, and the mechanical design for holding the Card. The MLM head electronics are controlled by peripheral registers known as status or data registers. Four registers are needed for the microprocessor to control and read the incubating reading head properly. They are head and well address register, emitter current drive (or D/A output) register, growth (or A/D input) register, and the control status register.

Each head forms a special purpose 60 channel densitometer operating at a wavelength of approximately 655 nm. The emitter board contains a buffer for the D/A analog signal, a modulation switch, and buffered decoding for the digital head and channel select signals. The buffered and modulated D/A analog voltage signal is converted to an analog current source which is switched to the proper LED by a double multiplexing scheme. The microprocessor has the task of providing the proper drive to the LED by automatic calibration of each head, when commanded. The detector board selects the proper phototransistor detector, converts the detected current to a voltage and provides a proper amount of amplification, thereby buffering it, and sends it to the growth board for conversion to a digital value usable by the microprocessor.

The incubation head and well address register, with decoding and multiplexed electronics in each head, determines which detection well will be read. The emitter current drive register determines with the digital to analog converter and emitter driver the exact amount of current the LEDs receive and, therefore, the amount of light emitted in the optical system. The growth register contains a digital representation of detector output as determined by the analog to digital converter and detector electronics.

The control status register controls the MLM head and mainframe electronics and informs the microprocessor of MLM status. Only one control bit is currently used and it is used to enable the timing and control circuitry which then enables the modulation and analog to digital converter. Status bits used by the microprocessor are: ready (A/D conversion complete), and light or dark modulation level.

The initial design of the head electronics, Figure 3-49, used eight 74145 BCD-to-decimal decoder/drivers for the emitter circuitry which typically dissipated 210 milliwatts per device. With the full system of five heads, approximately 8.5 watts of 5V power (i.e., 1.68 amps) would be required. The redesign, Figure 3-50, uses a double multiplexing arrangement thereby needing only two 74145s and, therefore, only approximately 0.42 amps, a savings of 75%. In addition, the detector circuitry was likewise simplified, and both emitter and detector printed wiring boards became less densely packed, therefore, the fabrication less critical.

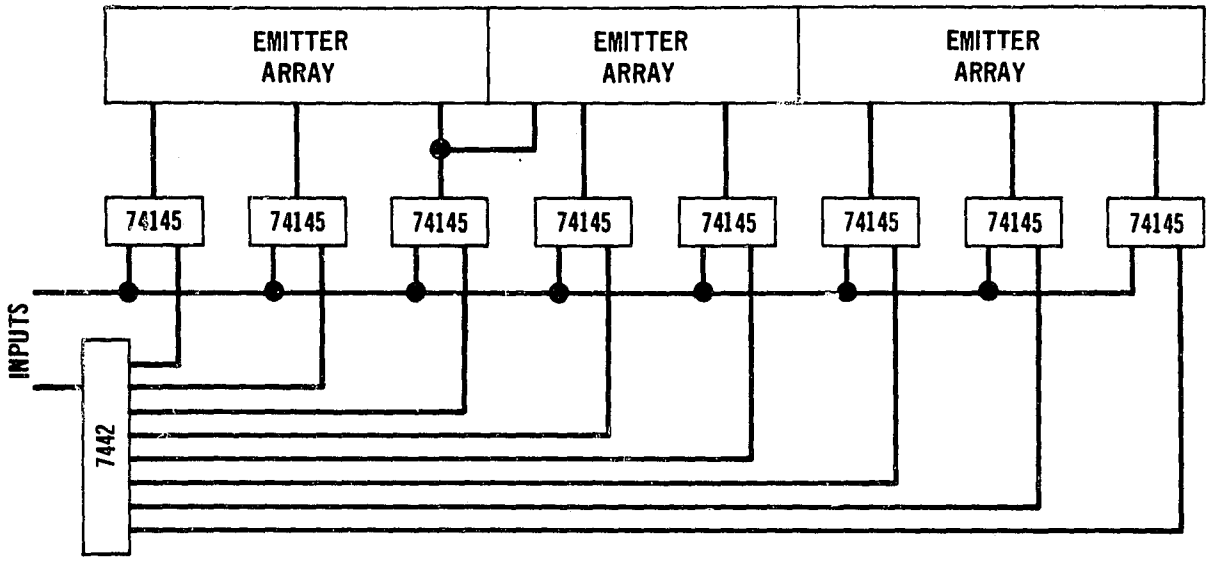


FIGURE 3–49
INITIAL MULTIPLEXING

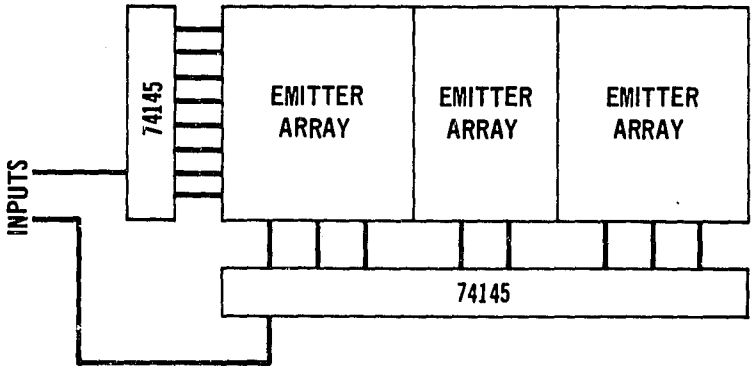


FIGURE 3–50
SELECTED DOUBLE MULTIPLEXING

Each emitter/detector array has 20 channels and is physically arranged in four rows of five channels each. Electrically each array is composed of five groups of four emitters/detectors. Grouping by fours allows closer matching to the 8 by 8 multiplex arrangement. The array is fabricated using unglazed Coors ADS-995 alumina substrate as the starting material. It is cleaned and metalization is evaporated onto the surface. It is then electro-plated with SEL REX 401 gold plating solution, rinsed, and dried. Photo resist is applied to the surface, exposed, and developed. It is then etched in three different solutions and rinsed between each etching solution. The remaining resist is stripped and the substrate is again rinsed and dried.

The plastic housing is machined from polycarbonate. Pins are inserted into the housing and bonded with Delta Bond 152-1-A epoxy and allowed to cure. The substrates previously prepared are cut to size with a diamond saw and the finished substrate is again cleaned.

The light emitting diode dies or the phototransistor dies are bonded to the previously prepared substrate with EPO-TEK H20-E silver epoxy and cured in a vacuum oven at 120°C for 30 minutes after all devices are mounted. Wire bonding of the dies is accomplished with a Hughes HPB-360 thermal compression bonder using 0.001 inch gold wire. The substrate is mounted in the plastic housing with BLH EPY150 epoxy. A milling machine X-Y stage is used in conjunction with an American optical binocular zoom microscope containing a cross hair reticle to align the substrate in the housing. The substrate is aligned in both planes and the epoxy is allowed to cure. The substrate conductors can now be wire bonded to the appropriate package pins and the array tested. When all units test good, the cover glass is cut to the correct size with a diamond wheel saw and the long sides beveled with an abrasive paper. The cover glass is cleaned and mounted with BLH EPY-150 epoxy, and clamped to the housing during curing. A completed set of arrays is shown in Figure 3-51. The LEDs are the smaller (labeled on the substrate as MLM-1) and the phototransistors the larger (labeled on the substrate as MLM-2) of the mounted devices.

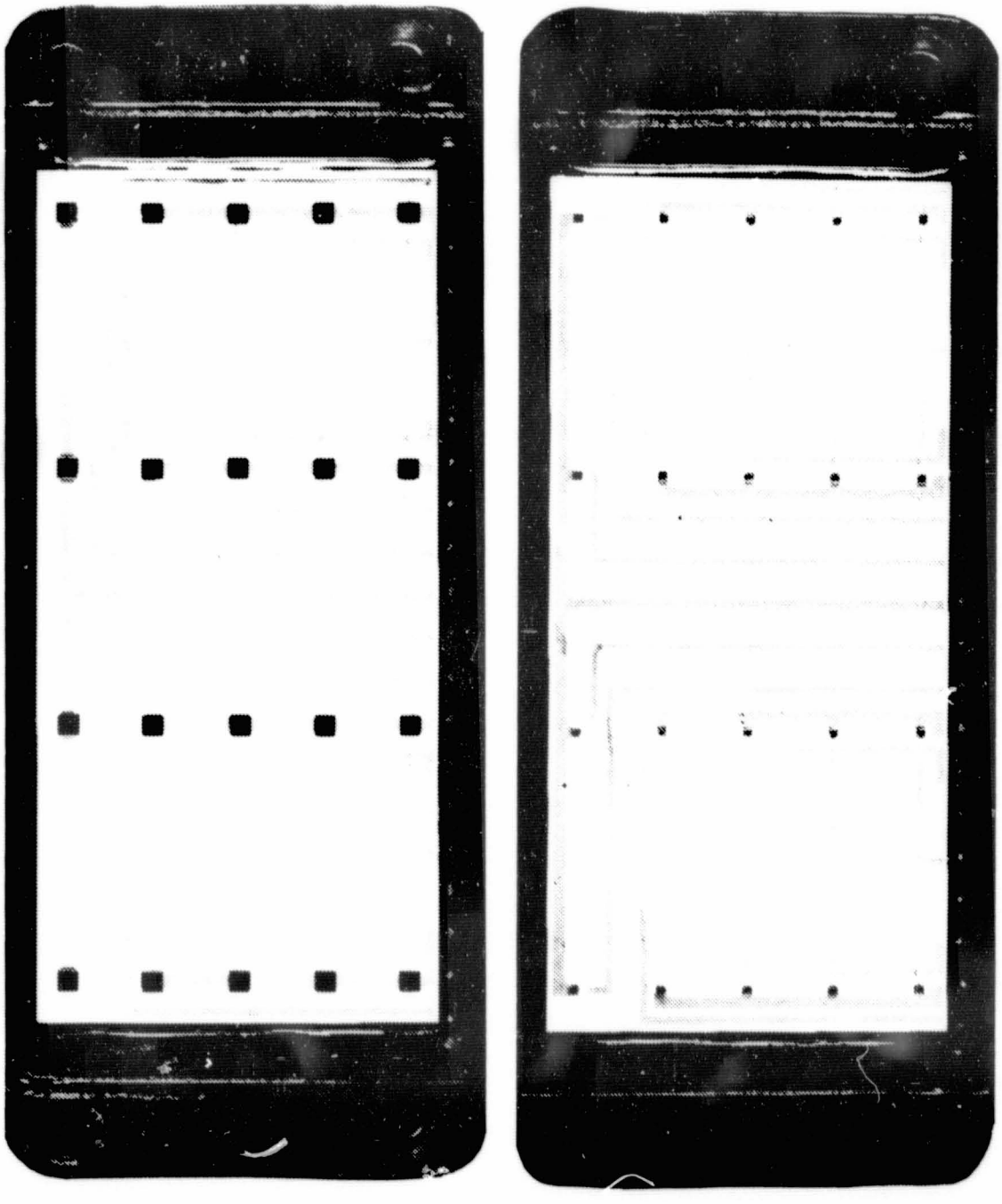


FIGURE 3–51
ARRAY SET

Incubator Design - Heat is provided for each side of the incubator head by five ceramic tube heaters. The three center ceramic heaters on each side are 3.35 inches in length and the two side heaters are 2.75 inches due to mounting constraints. Each ceramic heater, though, has an approximate resistance of 50 ohms and, therefore, a combined parallel resistance of approximately 10 ohms. The heater controller design makes use of 9 to 11 volt unregulated voltage to power the heaters. Heater input power may therefore vary between 8.1 watts and 12.1 watts.

Use of a 723 type voltage regulator in an unorthodox manner simplifies the temperature control circuit. The 723 has an internal voltage reference which is used to provide a stable voltage to the temperature sensing bridge with a thermistor located in one leg. The 723 also has an error amplifier which is used to amplify the voltage difference across the temperature sensing bridge. The amplifier output controls a medium current series pass transistor, included in the 723, which, in turn, controls an external power transistor.

The temperature monitoring circuit uses a separate thermistor bridge with 0.1% and 1.0% temperature stable resistors. The bridge output is buffered by two high impedance op-amps and is fed by multiplexers to the system's analog to digital converter. The microprocessor can, therefore, monitor each head temperature. With straight line voltage to temperature conversion, the head temperature can be read to within $\pm$ 0.3°C over a 20° to 50° centigrade temperature range.

Mechanical Incubator/Reader Design - Accurate and stable alignment of the Card and the emitter/detector pairs is important to accurate and reliable reading of growth. The incubator/reader head is a modification of the previous clamshell reading heads, with more accurate Card alignment than in previous units.

The Card is positioned between the emitter and detector mounting blocks as shown in Figure 3-52. Three emitter or detector arrays are mounted in each block as are the heating elements and temperature sensors. The array connector pins extend through slots in the array mounting blocks and mate with connectors on the printed wiring boards above and below the mounting blocks. Each array has two alignment holes and two screw holes which provides for accurate positioning and easy replacement of the arrays in the mounting block.

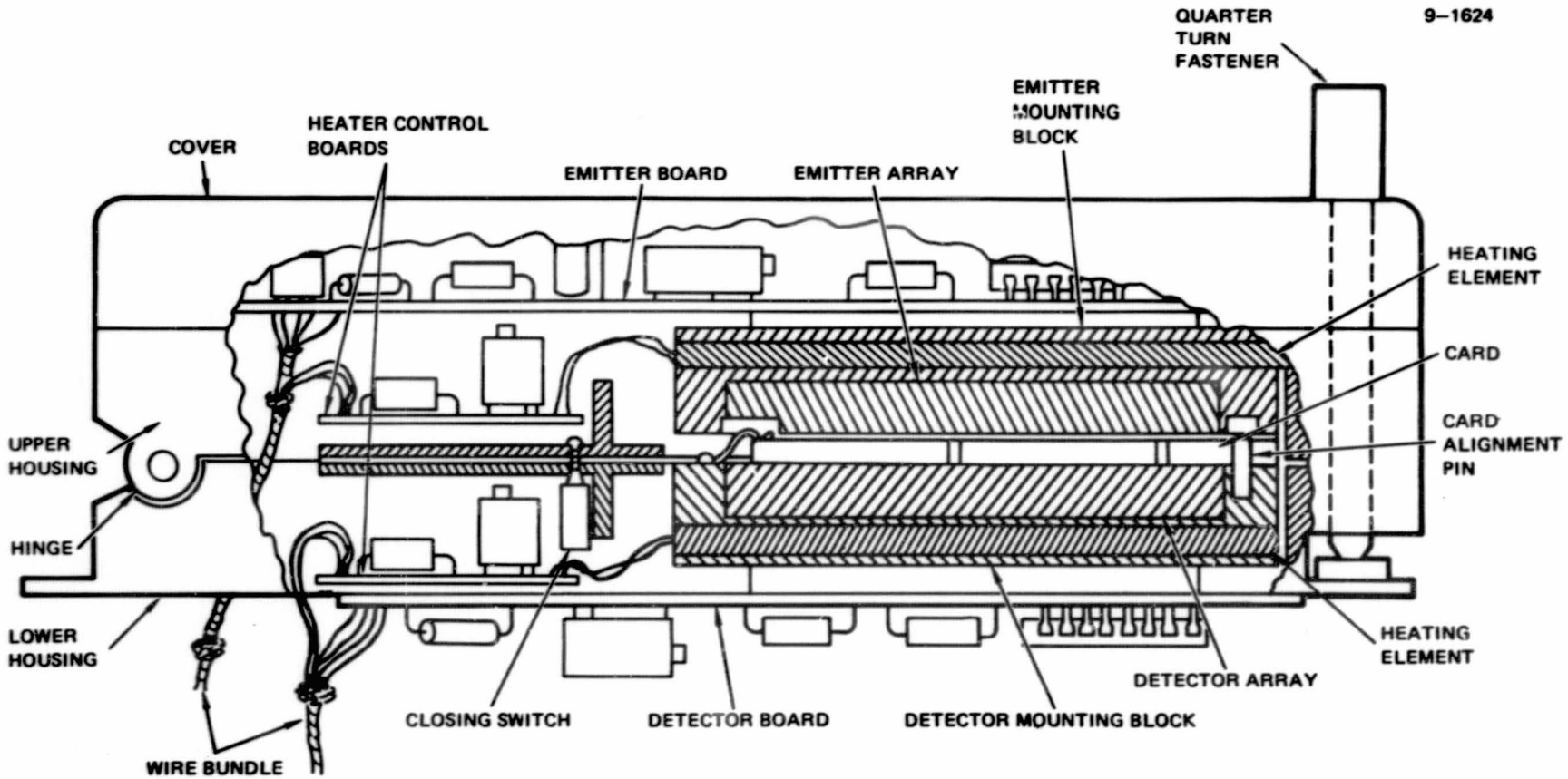


FIGURE 3–52
INCUBATOR – READER HEAD

The blocks are aligned with each other by using pins which are inserted through both blocks during assembly and then removed. Unless a mounting block is removed this procedure need not be repeated but can be checked at any time by using the alignment pins. The long clamshell pivot hinge provides for accurate positioning upon opening and reclosing. Distance between emitter and detector is assured by the positive quarter turn fastener.

The Card alignment between the array mounting blocks is controlled by two Card alignment pins at the front of the head. These pins are offset so the Card can be placed in the head in only one orientation. Small leaf springs hold the opposite end of the Card flat against the detector mounting block.

3.2.3.2.4 Interfaces - Four peripheral interfaces are necessary for the microprocessor to interface with the operator and provide time history plots of the large amounts of data gathered in the 13 hours necessary for each test. The five interfaces (3-1/2 of which are located on the interface board) are serial interface (Teletype), parallel interface (magnetic tape recorder), keyboard interface, display logic, and Sample Loading System control. The SLS control is located next to the Sample Loading System and the latches for the display are located on a board next to the alphanumeric character display.

The serial port could have been implemented two different ways using the IMP-16C microprocessor. In one method the serial port could use the flag and sense lines provided on the microprocessor board or, in a second method, additional logic could be used to decode a device address and latch a single bit from the RO register. The second method was used since many device addresses would be used throughout the MLM and could not be accommodated with flags and sense lines alone. The device address decoding would keep all of the addressing schemes the same. Timing of each bit is still by microprocessor software timing loops. Incoming 20 milliampere serial data is limited, buffered, and converted to TTL compatible levels and read by the microprocessor which converts it to a parallel word or byte (8 bits). Outgoing serial TTL levels are converted to a 20 milliampere current loop compatible with the ASR 33 or other low impedance devices. High impedance devices could reduce the 20 milliampere current since only a low voltage is used in the interface.

The parallel port has eight data lines out, eight data lines in and seven handshake signal lines. The output lines are latched and buffered to drive twisted pair lines. The input lines are conditioned and tristate buffered to the microprocessor. Data is assumed to be held externally until the next read command is given. The seven handshake signal lines are: outputs, RC - Read command, WC - write command, inputs, RE - read enable, WE - write enable (opposite of RE), RRDY - read ready, DA - data available (nearly the same as RRDY), WRDY - write ready. The transfer rate of the parallel interface is limited by the microprocessor and the external device.

The serial port interfaces to a Teletype purchased from Teletype Corporation, and the parallel port interfaces to a magnetic tape recorder purchased from Electronic Processors, Inc. The Teletype is a Model 3320-3JC with keyboard and printer mechanism. It uses the standard ASCII code with only upper case letters. The punch mechanism for auto-on and auto-off has been disabled. The reader power control has been modified to allow microprocessor control. The magnetic tape recorder is an unmodified STR-200 for continuous character recording. All formatting (blocks, records, gaps, etc.) is under microprocessor control. The operator has control of reading, recording, rewind, fast forward and tape eject functions. The Phillips type digital cassette uses tape without clear leader at the beginning of the tape.

The keyboard is purchased from Cherry Electrical Products and includes an ASCII encoder. To interface to the microprocessor only tristate buffers are required. Two bytes are read into the microprocessor and reformed with software to one eight bit byte and a status bit.

The alphanumeric display is a peripheral device with its own memory. One memory location is allotted to each character position of the 16 position display. Timing chains composed of binary counters provide the scan rates for row scan (seven of eight time periods), position character load (16 out of 256 time periods), and multiplex (update from microprocessor allowed). The ASCII character and the row information becomes an input to a decoding ROM which then provides five dots of information to each character position in turn. When all 16 positions are loaded (five dots each) into the latches the row is displayed. This continues at a flicker free rate for all seven rows.

Power Supplies - The power supplies selected for the flight prototype version of the MLM are 60 Hz, 120 volt input supplies from Standard Power, Inc. The main supply, the SPS-250T, is a triple output series regulated direct current (d.c.) supply. Output voltages are: (a) five volts at 12 amps maximum, (b) 15 volts at 3 amps maximum, and (c) -15 volts at 3 amps maximum. The secondary supply, the 200B7, is a 20 amp maximum unregulated d.c. supply at a nominal voltage (half load) of 10 volts. Other voltages such as -12 volts, -5 volts, and +5 volts for the alphanumeric display use regulators near the point of usage.

For low noise, single point grounding was used wherever possible. Also separate power and ground wires are used for the digital voltages, analog voltages and unregulated voltage.

3.2.4 Ancillary Equipment - Throughout the development of the Microbial Load Monitor some ancillary equipment has been necessary for the fabrication of cassettes/Cards and their loading devices. Some items have been as simple as an X-acto knife and needed no design work or development. Others such as media loaders or Card tapers did require designs for each type of media or cassette/Card used.

The first major design work on ancillary equipment in this contract was for a filter/media cassette punch. This was used to cut and load filters or plastized media into the cassette. All necessary filter or media discs for one cassette could be loaded simultaneously.

The punch, pictorially shown in Figure 3-53, is operated by a small arbor press. Each punch cuts a round disc slightly larger than the detection or filter well in the cassette. Inside each punch is a pin which presses the cut filter or media disc out of the die block and into the cassette. The pins also compress the filters to a controlled thickness. Alignment pins in the base insure the proper location of the cassette. A photograph of the punch in operation is shown in Figure 3-54. Some difficulty was experienced with this device during the program, resulting in several modifications and a new design approach. Lack of consistency in filter density and poor placement in the filter well were the greatest deficiencies.

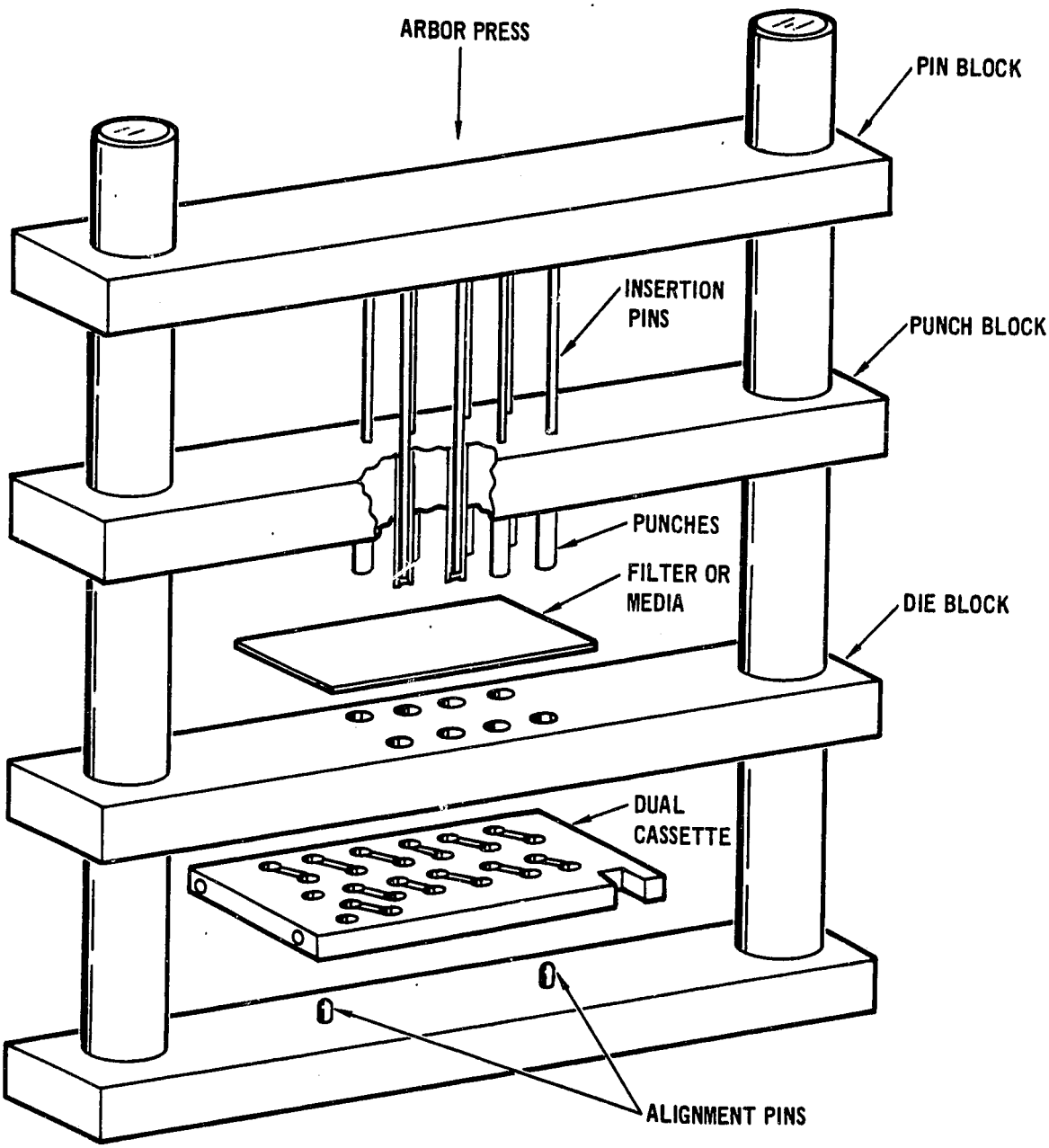


FIGURE 3–53
CASSETTE LOADING PUNCH

9–1664

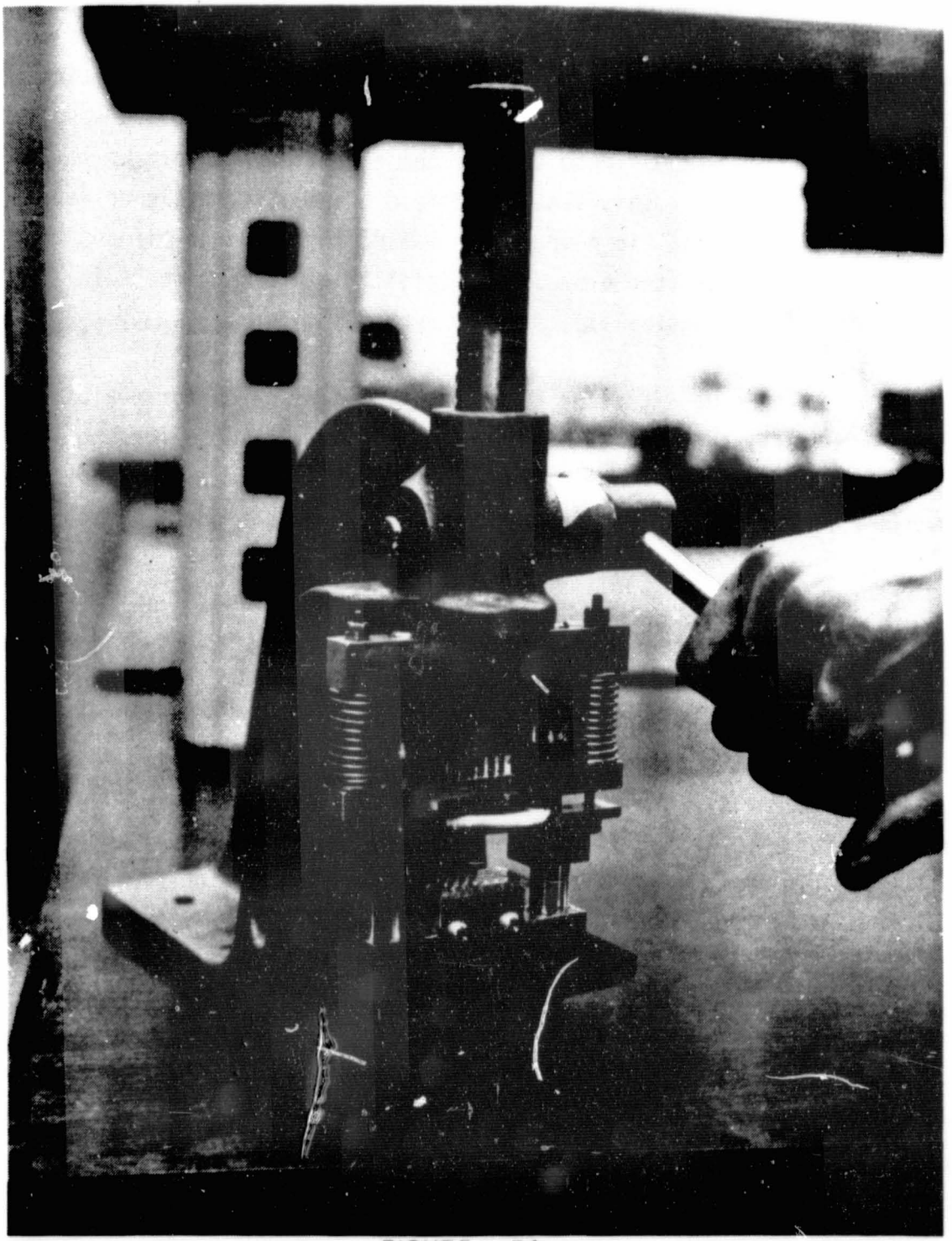


FIGURE 3–54
CASSETTE LOADING PUNCH

The Card assembly process, at this point in time, involved a series of tasks including washing and sterilization of the molded plastic blank, media loading, freeze drying and Card taping. Sterilization of the molded plastic blank was subsequently dropped but the washing and cleaning procedures tightened. A critical operation in this sequence is the consistent media loading procedure. To resolve this problem area, a system was designed to have the capability to load up to 20 different media simultaneously. This system utilized a specially designed feeding head and a multi-channel peristaltic pump which metered the required aliquot of media into the Card in a three-step process. Initial trial loading tests in the laboratory demonstrated the feasibility of this process.

Hand taping units were used to tape small quantities of Cards for evaluation testing. These were sufficient for the few freeze dried Cards taped for the limited initial testing performed with the 60 well Card. A higher rate machine, though, would be necessary to produce the 100 to 1000 Cards in a media production run.

3.2.4.1 Taping Unit for the Flight Prototype Card - The present version of the taping unit was designed to reduce taping operation time and to ensure consistency in tape tension and application pressure. The Cards are fed into the machine from a hopper while the operator pushes a lever to force the Cards through the machine, Figure 3-55. At the first station the Teflon tape is laid lightly onto the Card. At the second station, the tape is rolled under high pressure. At the third station, the operator cuts the tape with a knife through guides. With this machine, one person will be able to tape one side of 300 Cards per hour. The machine saves a considerable amount of tape compared to the manual hand taping device previously used and improves the consistency of the tape bond to the Card.

The original design of the taper was modified because of a change in the tape used on the Cards. The original machine was designed to use 3M manufactured tape that did not have a liner. Due to problems with this tape and the reluctance of the manufacturer to continue to market this product, it was decided to find another source. We are now procuring this tape from Fasson. It is chemically the same as the 3M tape with the same adhesive but comes with a release liner. This eliminated the problem of tape sticking to itself and made it easier to apply. However, the taper had to be redesigned to add a take-up reel for the liner. Other than the addition of this take-up reel, the taper, Figure 3-56, is basically the same as the

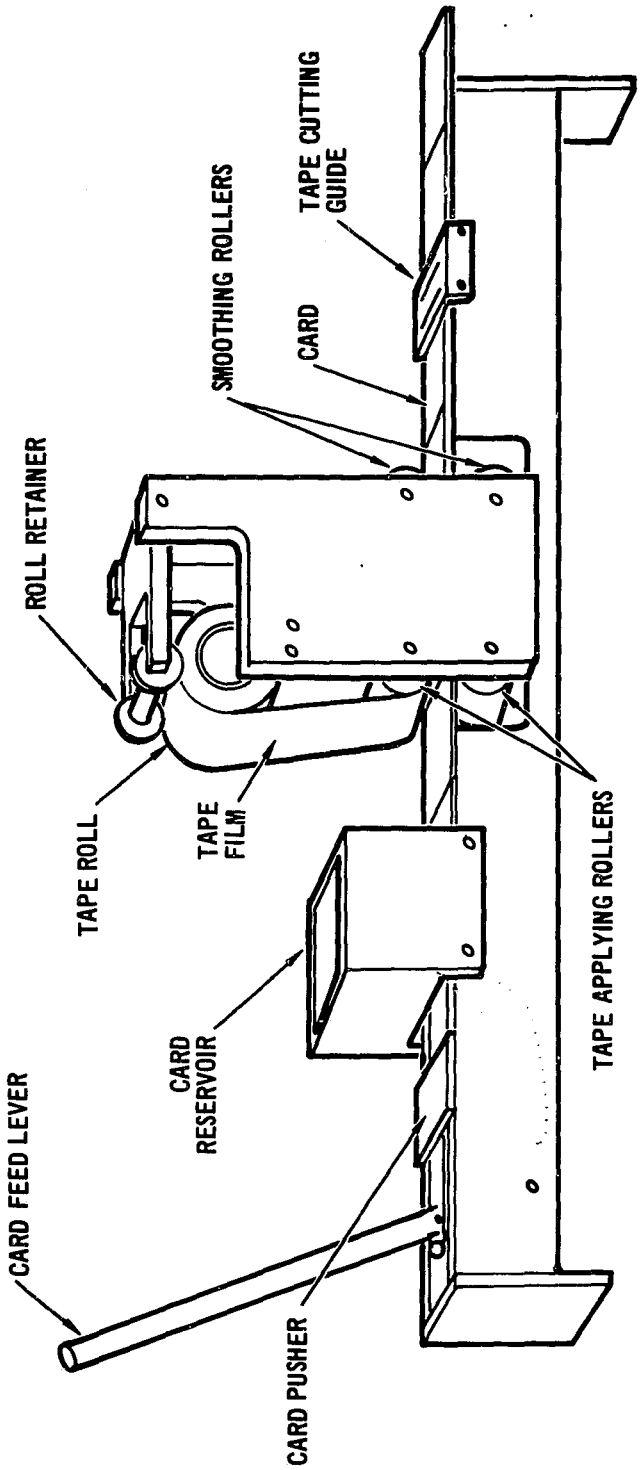


FIGURE 3–55
MLM CARD TAPING MACHINE

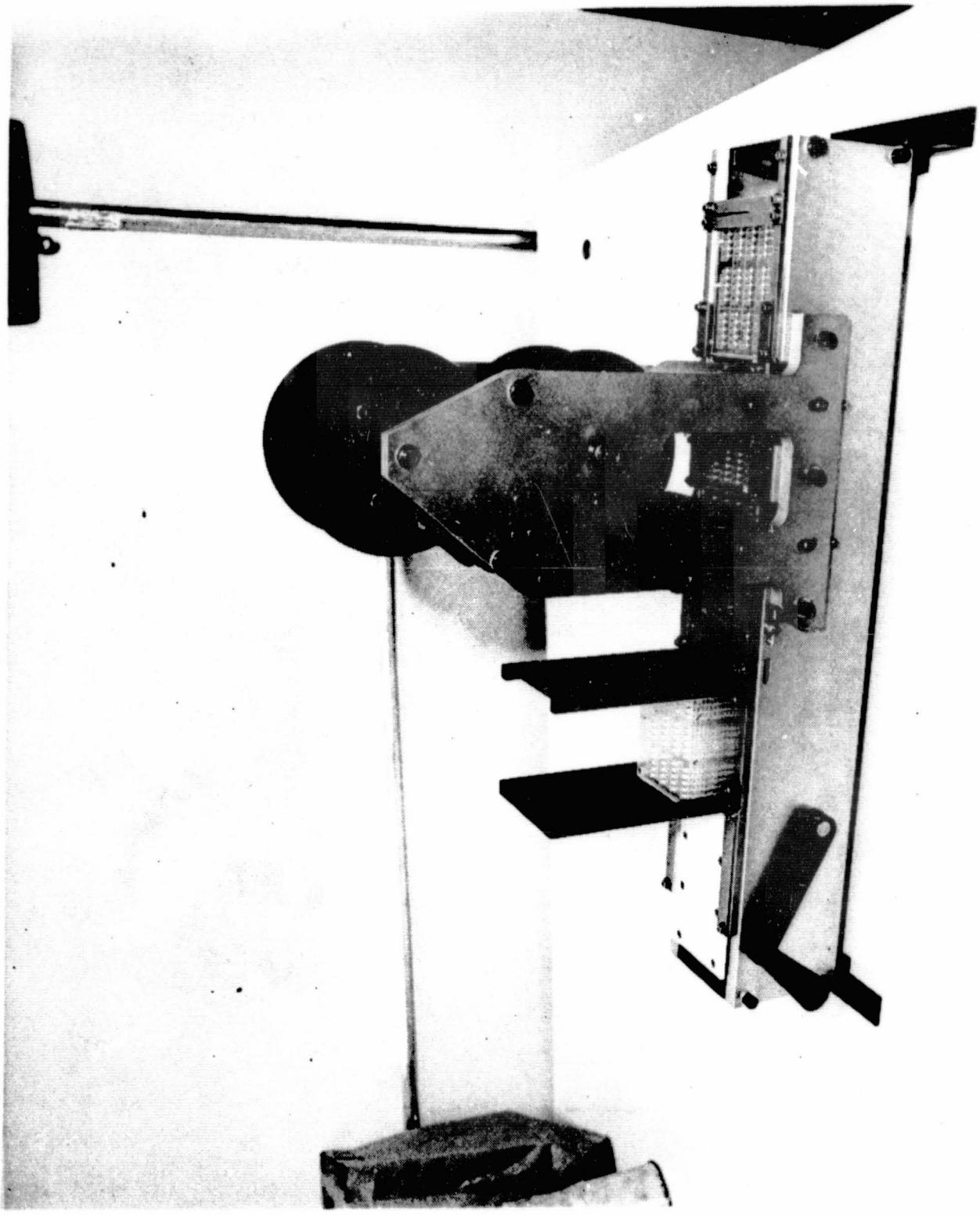


FIGURE 3–56
CARD TAPER UNIT

original design. The rear view, Figure 3-57 shows the take-up reel on top and the slip clutch necessary to achieve liner tension.

3.2.4.2 Media Pumping System for the Flight Prototype Card - The media pumping system for the 60 channel Card as shown in Figure 3-58 is comprised of two units, the media pumping station and the dispensing station. This pumping system can dispense up to 60 different media formulations simultaneously to 60 wells.

Each pumping channel contains a Hamilton microliter syringe, a 20 gauge needle attached to the syringe, tubing, and a 20 gauge dispensing needle. The media pumping station also contains the motor, cams, and switches to drive the pump as seen in Figure 3-59. The media dispensing station, Figure 3-60, contains the dispensing head, the media reservoir, a tray with handle for holding the Cards, a manual crank for raising and lowering the dispensing head, and control switches.

The original design called for the dispensing head to translate from the media holder to the Card horizontally. There was some concern that during this translation media of one type may accidentally drop off the dispensing needle into another media reservoir, thus contaminating it. This translation action was eliminated and replaced by movement of the Card and associated Card holder.

Each channel is calibrated gravimetrically for the desired amount of media. This ranges from 17 to 20 microliters with a tolerance of $\pm$ 2%. Calibration is accomplished by dispensing 10 drops of distilled water, weighing them on a scale and adjusting the bolt at the top of the pumping unit until the desired results are obtained. Each microliter syringe is marked and used for only one media. All plungers are pulled to the same height and the bolt determines the low end of the stroke.

To operate this system, each pumping channel must first be filled with proper media so that there is no air in the system. This is done by first elevating the dispensing station above the syringes so that media will flow from the dispensing needle to the syringe. With the needle at the end of the syringe disconnected from the syringe and the plunger completely depressed, media is allowed to flow until it flows out of the syringe needle. The needle is then connected to the syringe, and plunger completely pulled out, and when all the air is displaced by the media the

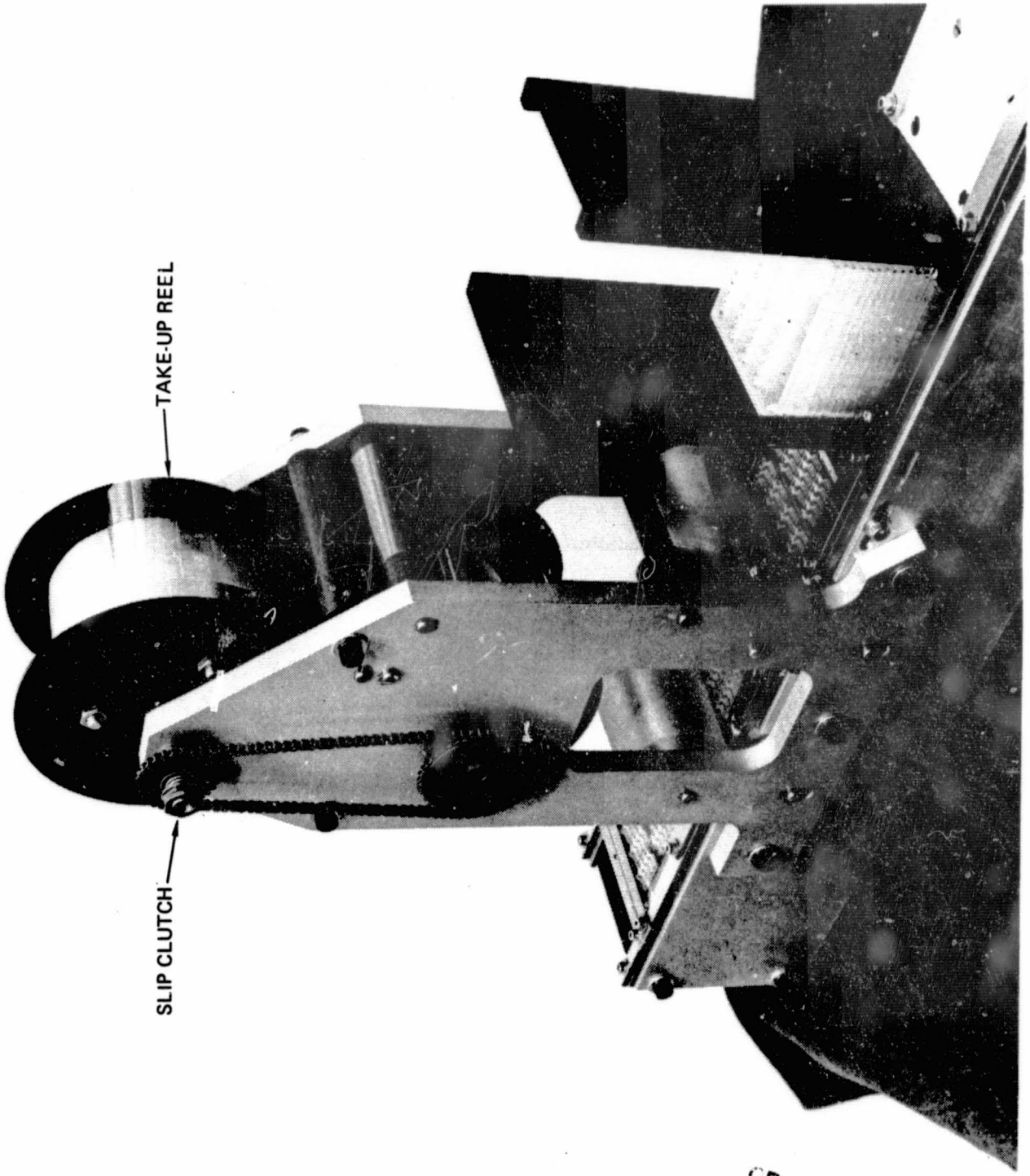


FIGURE 3–57
CARD TAPER UNIT (REAR VIEW)

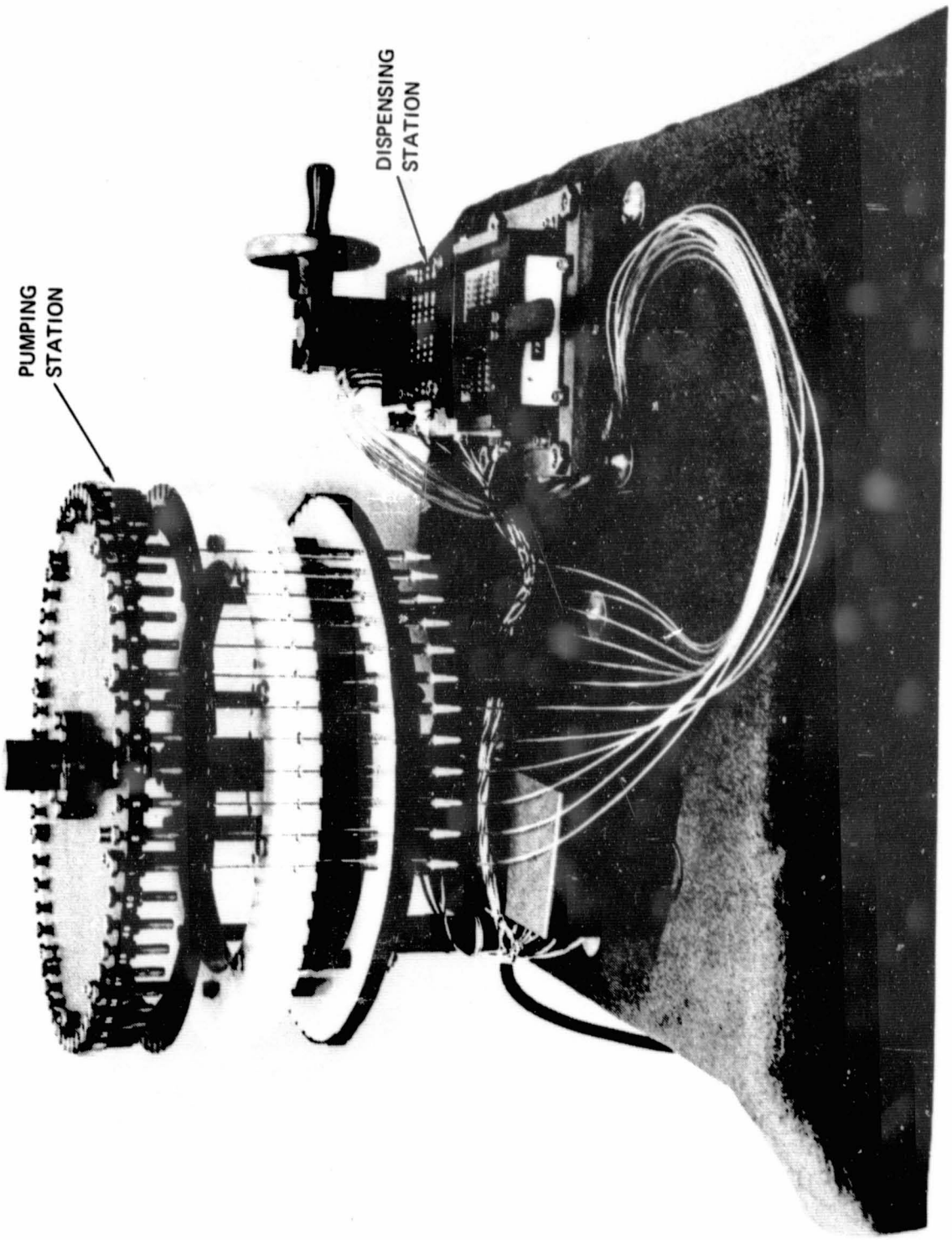


FIGURE 3–58
MEDIA PUMPING SYSTEM

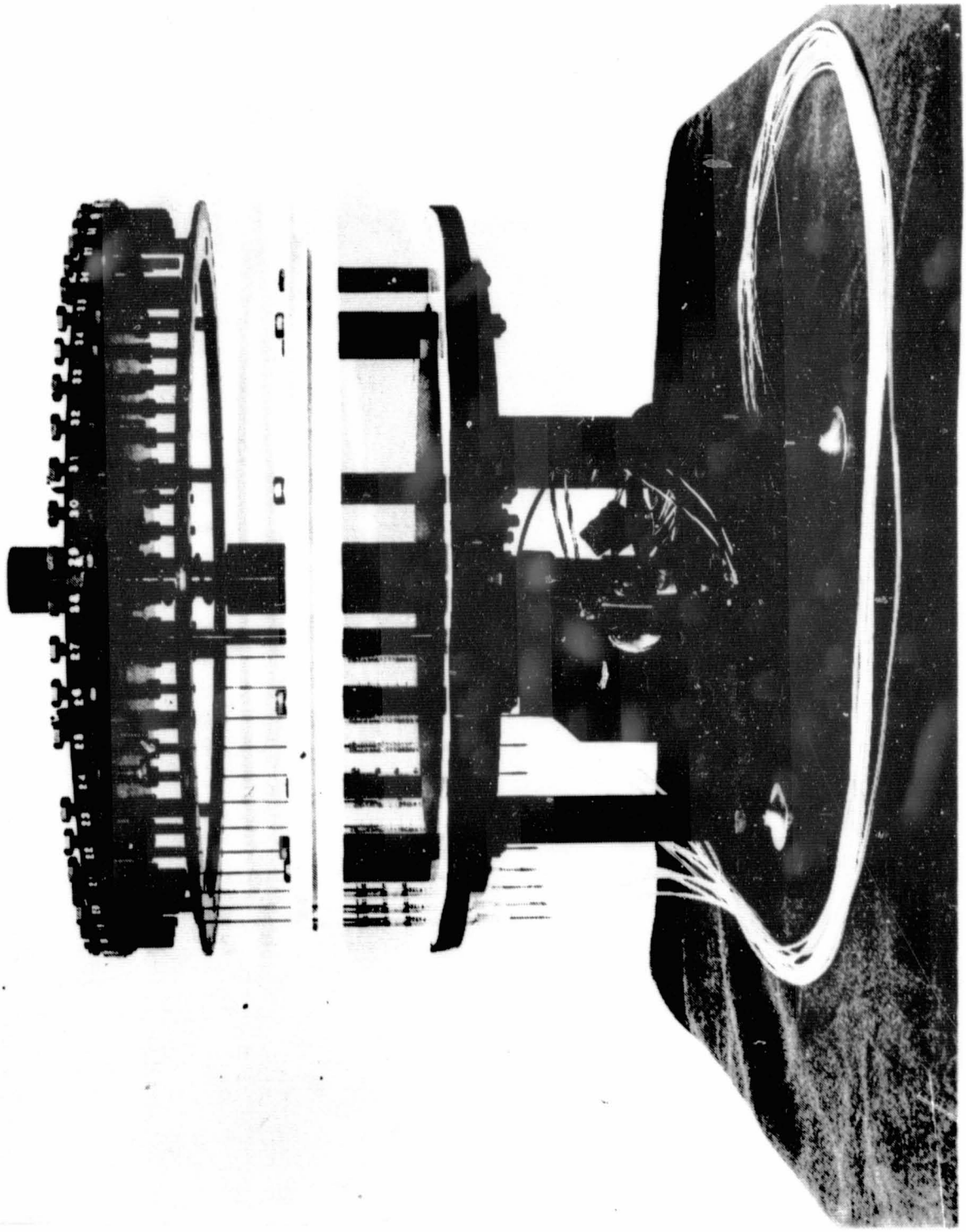


FIGURE 3–59
MEDIA PUMPING STATION

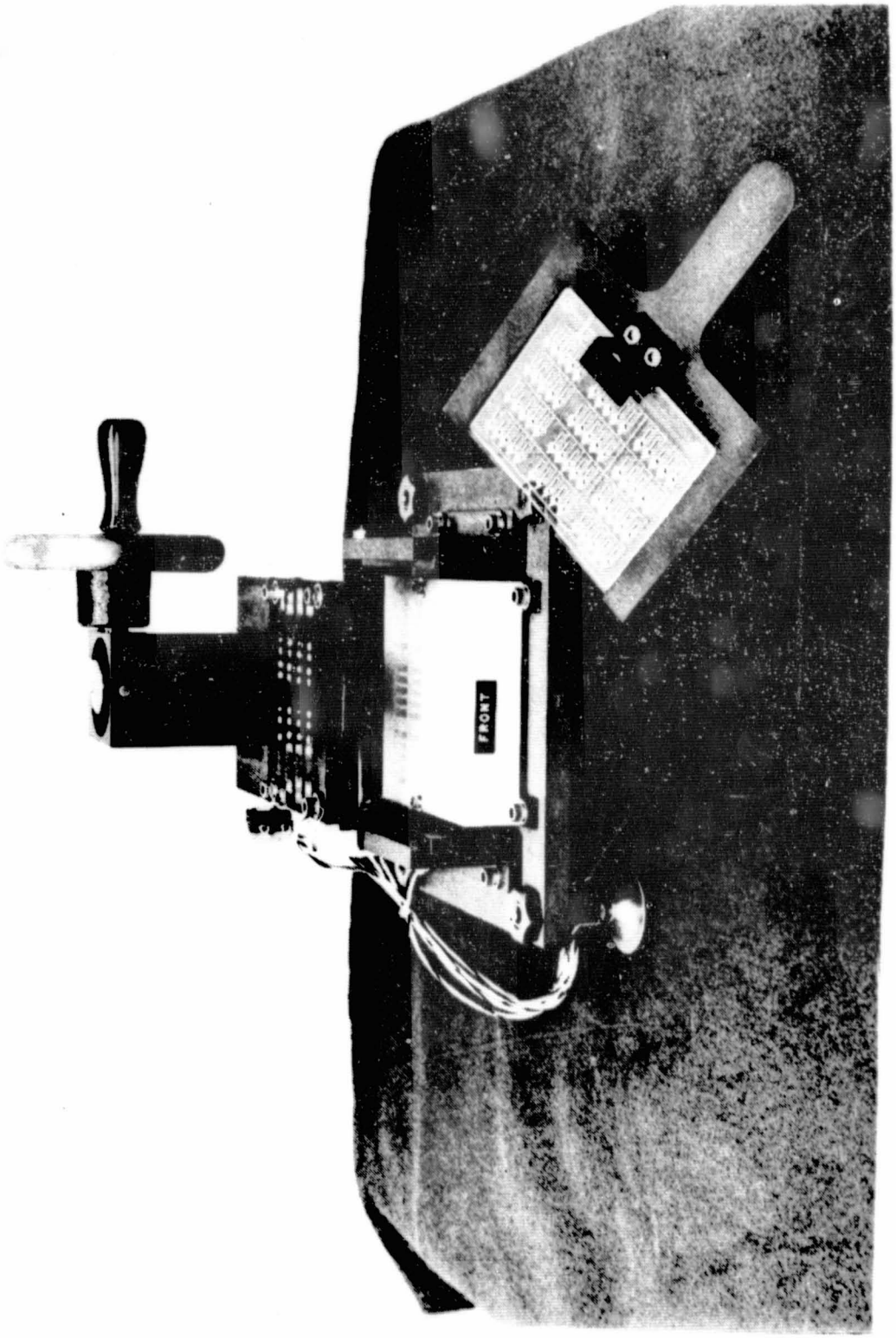


FIGURE 3-60
MEDIA DISPENSING STATION

plunger is reinserted into the syringe. This is done for all the channels required for the particular Card to be filled. When this purging operation is completed, the dispensing station can be lowered to its normal height and the filling of Cards can start.

A Card is filled by first placing it onto the Card tray. The Card tray has two dowel pins which fit in the Card alignment hole to aid in lining up the Card with the dispenser and prevents the Card from being put into the tray in the wrong orientation. Before inserting the load tray into the dispensing station, the dispensing head is lowered until a mechanical stop is reached. This inserts the dispensing needles into the media and activates a microswitch which energizes the pump motor. All the syringe plungers are pulled up sucking the media into the needles. The head is then hand cranked to its uppermost position, the Card tray inserted and the dispensing head lowered until it hits the stop on the tray. The tray insertion activates one switch and the head another. This combination energizes the pump motor again and the syringe plungers are pushed down dispensing the proper amount of media into each hole. This action is repeated until the desired number of Cards are filled. One media reservoir can fill approximately 40 Cards. Additional reservoirs are used for additional Cards.

3.2.4.3 Sample Receiving and Card Loading Device Fabrication - Very early models of the loading devices were completely handmade, one of a kind test items. The first loading device produced in any quantity was described in Section 3.2.2 and shown as the diluent cartridge in Figure 3-22. This cartridge needed a drilling guide for placement of the needle hole in the lower portion of the device. The rest of the assembly involved cutting the diluent tube from round stock and glueing the needle (inserted to the proper length), tube, body, and bottom together. The unit was tested for leaks and cleaned.

Fabrication of SRCLDs is more complex and time-consuming than the early diluent cartridges which were designed only for a one-g earth environment. There are two reasons for this: (1) the SRCLDs are composed of many individual pieces but the total quantity of SRCLDs fabricated is too small for extensive automation, and (2) the quality control of fabrication must be excellent to minimize three possible problems. These are needle clogging, SRCLD diluent leaks, and intermittent impeller rotation. Figure 3-61, Sample Receiving and Card Loading Device,

9–1627

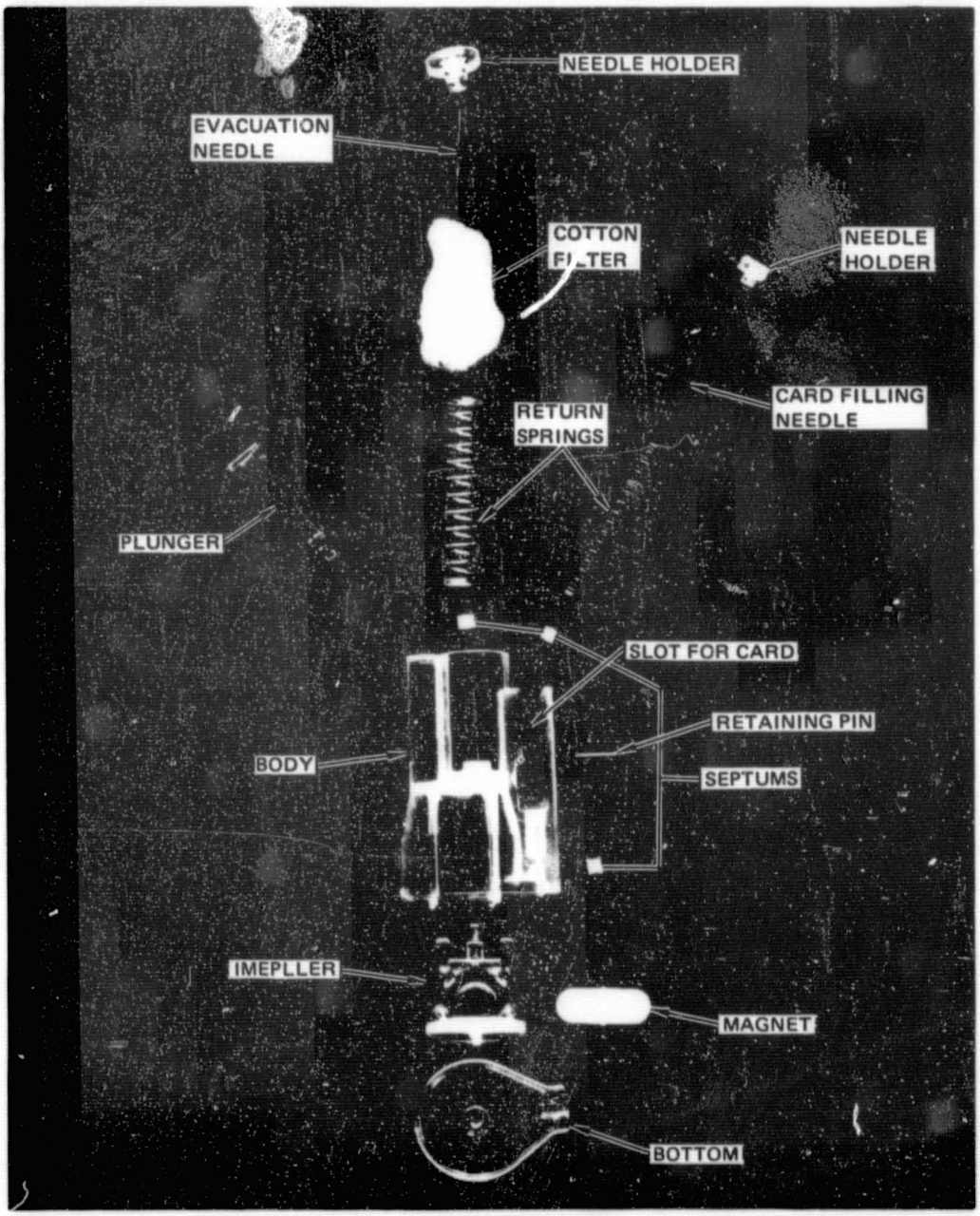


FIGURE 3–61

SAMPLE RECEIVING AND CARD LOADING DEVICE

shows an expanded view with all parts labeled. One additional part, the rubber tip for the plunger, is now shown and is identical to the rubber plunger tips of small syringes.

There are five special items necessary for the assembly of SRCLDs. The items and their purpose are described below in the order of their use. First the filling needle fixture is used for depth adjustment of the filling needle in the small needle holder. The second item is an aluminum-Teflon tip inserted in a 27 watt #537 Ungar soldering cartridge and is used to form a flange on the SRCLD body so that the large needle holder will remain with the SRCLD. The third item is a fixture shaped to hold the SRCLD body during the above flanging operation. The last item is a sonic welder and shaped horn which is used to weld the bottom to the body after all items have been assembled except for the plunger and the liquid specimen septum (located near the bottom piece when assembled).

Quality control to prevent needle problems is concerned with inspection of the hole for the retaining pin for proper placement. This hole is drilled by the molded part supplier. Improper placement can cause the filling needle to jam and bend during the loading operation and as a consequence the Card cannot be filled. The amount of septum material injected into the filling septum area can also influence the filling of the Card. Too much material could surround the needle tip and prevent the evacuation of the Card. Coring of septum material could also clog the needles so each needle has an anticoring bent tip to minimize this problem.

Leaky SRCLDs are found by using a 50 ml syringe with a small needle, through a rubber plunger tip. The SRCLD is pressurized through the solid sample port and held under water. Bubbles are apparent around any leak. Leaks can sometimes be resealed with Pleximent or by carefully using a clean soldering iron to locally melt the plastic in the area of the leak. The preferred and the more reliable method is use of the soldering iron.

Impeller rotation is influenced by the pivot points of the impeller, the tightness of fit of the magnet, centering of the magnet (so it does not rub the diluent chamber walls), and the magnetic strength of the magnet. All four areas should be examined during assembly for minimum rotational problems.

3.2.5 MLM Software - Early models of the MLM had no need for any sort of software since they were dedicated analog instruments and continually took readings. With the advent of the digital units (specifically the 100 channel, carousel, and flight prototype models) some thought had to be given to software use.

3.2.5.1 Programmable Calculator Software - The first software was written for the Wang 620-B calculator. The 100 channel MLM was monitored by the programmable calculator since the quantity of data was too great for hand monitoring and time history profile plotting. The Wang 620-B had no direct control of the instrument but could only monitor its sequential operation. With data storage a problem, calculations, and time history profile plots were performed in real time. Continuous loop paper was used to simplify software.

With the addition of the carousel unit the continuous loop method of plotting became unweildy. The Wang 620-B was upgraded to a 620-C and a dual magnetic tape cassette unit was added for data storage. Data gathering and time history profile plotting were now performed separately. The programs for the carousel version of the MLM were modified and used at NASA JSC during Spacelab simulation tests with the first 50 channels of the 100 channel instrument. The Wang 620-C and related equipment performed the function of remote data gathering and time history plotting. The results were good.

3.2.5.2 Flight Prototype Software - The software for the Flight Prototype MLM is logically divided into two sections, that which acquires data and that which plots time history profiles from the acquired data. In the initial versions, these were two separate programs with some common subroutines. The programs were initially separate due to their combined length and the amount of memory available in the MLM. In an engineering system, more random access read/write memory (RAM) is used than is needed in a dedicated system. Likewise the reverse is true with PROM or ROM (read only type memories). This facilitates changes in programs. Physical constraints limited the MLM to 4K of CMOS RAM and 1K of PROM. (The abbreviation K when used with computers, or software memory requirements typically refers to 1024 rather than 1000 as in most other usage.) After the engineering and development was complete, one 2K CMOS RAM board was replaced with an 8K PROM board. The capability of the final memory configuration is thus 8K of PROM and 2K of RAM

The IMP-16C microprocessor when initialized starts at location FFFE (hexidecimal). Start-up routines are, therefore, placed in PROM at this starting address. A hardware restart can be commanded by simultaneously depressing the break and reset keys. A module representation of MLM software is shown in Figure 3-62. These eight modules and the Monitor configure the present MLM software.

The Monitor module as shown is the center of all action. Its two basic functions are to monitor the keyboard and to monitor time as read by the Clock module. All other action is initiated by the instrument's operator. The Monitor decodes all requests and branches to the appropriate subroutine or module to perform the request. It then supervises the action until completed, i.e., it re-enters the subroutine or module for multiple readings based on time. The Monitor will also make checks to see if a request is allowed. Examples would be: If an incubating/reading head is still within the 13 hour incubation period, a request to plot time history profile would cause the message HD ACTIVE: likewise, a request for final status during the 13 hour period would cause the message INCU NOT COMPL. The main operator requests are four letter commands. The operator's manual lists the commands and explains them in detail. Table 3-7 lists the commands with an abbreviated explanation.

The Monitor module also includes various math subroutines and driver subroutines. The IMP16-C has commands for multiply and divide but are only unsigned numbers (positive only). The math routines include percent change calculations, and signed multiply and divide. Additional general subroutines are binary to binary coded decimal (BCD) conversion, hexidecimal and decimal validity checks, and message output subroutines. Outputs are set up so that many peripherals can be changed by changing the associated driver. Drivers included in the Monitor include one for display and keyboard, one for a 110 baud ASCII serial port, and one for the formatted data of the magnetic tape parallel port.

The Clock module has the function of controlling and reading the clock integrated circuit. This module initializes the clock (month, day, hour, and minute). with inputs from the operator. The time read can be used by the Monitor module to compute elapsed time for each head and decide whether to read or not to read based on predetermined time intervals. The clock is configured to generate a 24 hour clock. Initialization commands are ITIM and IDAT. The time request command is TIME.

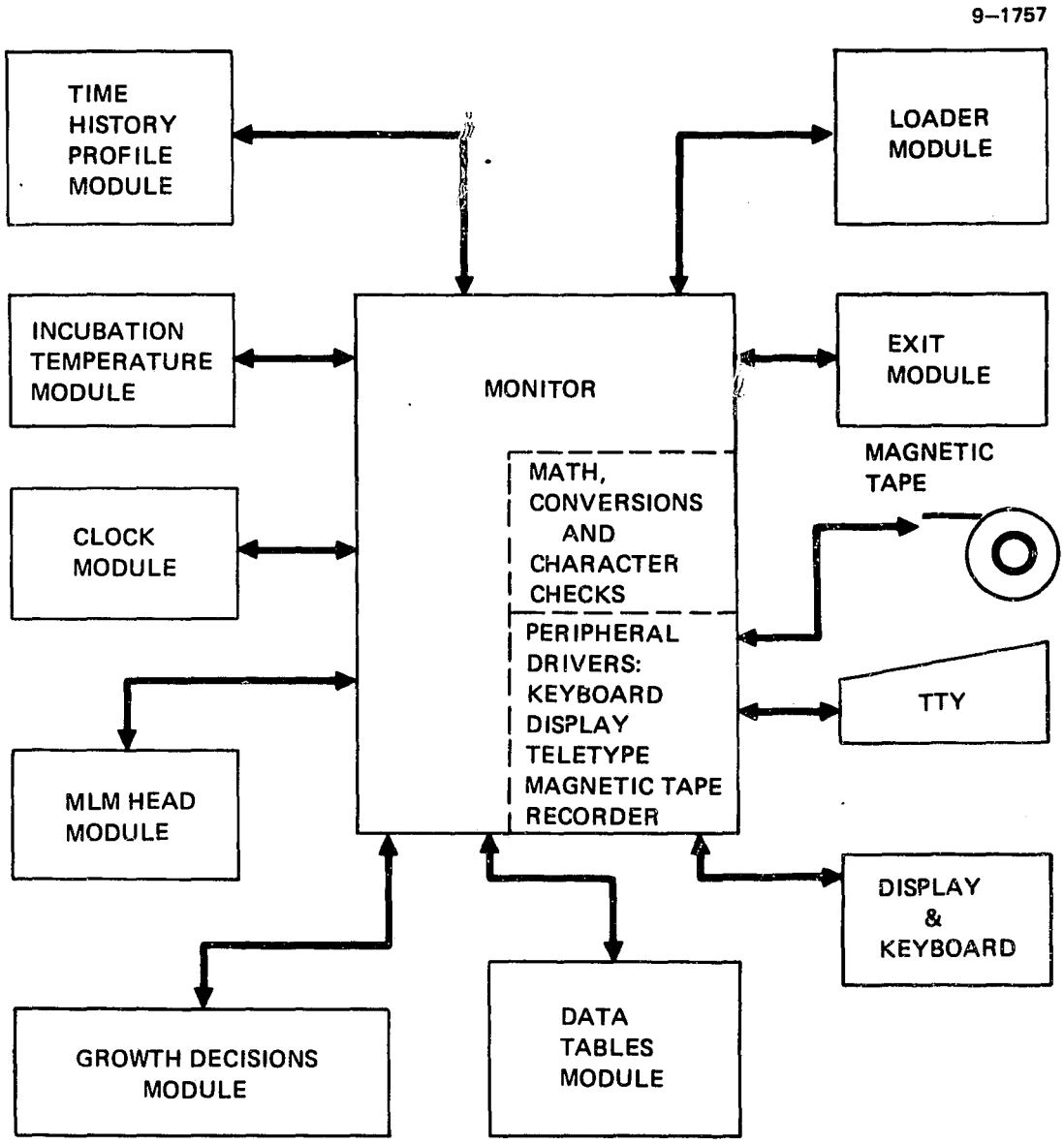


FIGURE 3–62
MLM SOFTWARE ORGANIZATION

TABLE 3–7

MONITOR COMMANDS

9–1825

CALH	– CALIBRATE THE LED/PHOTOTRANSISTOR PAIRS
READ	– READ INCUBATING/READING HEAD WITH ZERO ELAPSED TIME INITIALIZATION
ITIM	– INITIALIZE THE CLOCK TO INPUTS(HOUR AND MINUTE)
IDAT	– INITIALIZE THE CLOCK TO INPUTS (MONTH AND DAY)
LOAD	– OPERATE THE SAMPLE LOADING SYSTEM FOR LOADING A CARD
STAT	– REQUEST THE CURRENT STATUS OF A PARTICULAR READING HEAD
FINS	– REQUEST THE FINAL STATUS OF A HEAD (VALID ONLY AFTER 13 HOURS)
TIME	– REQUEST THE DATE AND TIME AS KEPT BY THE CLOCK
PLOT	– PLOT TIME HISTORY PROFILES
TEMP	– REQUESTS THE TEMPERATURES OF A PARTICULAR HEAD
EXIT	– EXITS THE MONITOR MODULE AND ALLOWS SPECIAL COMMANDS AND/OR PROGRAMS TO BE RUN
INIT	– INITIALIZE BASIC RAM MEMORY LOCATIONS WHEN FIRST STARTING

The MLM Head module has the function of addressing each head and reading the detected light level of each channel. The raw data is sequentially stored in the Data Tables module. One section of the MLM Head module has the capability to calibrate the light emitting diodes' drive currents when the Monitor is commanded to do so by the operator. The associated commands are READ and CALH. A module subroutine requests which head by displaying a "#" character symbol on the alpha-numeric display. The inability to calibrate one or more LEDs triggers the message CAL ERROR.

The Data Tables module is the largest of all the modules since all data resides here. A complete MLM system will need data room for 300 initial values, 300 present data values, 300 different light emitting diode current drive values, 5 Card starting times, 120 threshold values, and space for all organism names, fault notification and operator commands. This is a distributed module - meaning a portion resides in RAM and other portions reside in PROM near the related modules. Also it is not directly accessible by the operator. Only by exiting the Monitor (EXIT) can individual words be examined.

The Loader module, when commanded by the operator, controls the Sample Loading System. The four letter access command is LOAD. From this point, the Loader module interacts with the operator to load one Card from two or three SRCLDs. One letter commands are used: to continue (Y), to abort (Q), to reramp (M), and to reramp the magnet motor to a lower speed (D) or higher speed (U). The module returns to the Monitor with the displayed message LOADED.

The Incubation Temperature module is used by the Monitor to check incubation temperature of each side of an active head. If the temperature is within preset limits, then a normal return is taken to the Monitor. If either temperature is not within preset limits of $\pm$ 1 degree centigrade it triggers TEMP ERROR HD #N message where N is the head number. The head read subroutine also includes a check for temperature error limits of $\pm$ 2 degrees centigrade which when exceeded will abort the test in that head and print on the teletype the message, "END OF HEAD READING DUE TO CAL AND/OR TEMPERATURE ERROR!" Temperature can also be requested by the operator with the four letter command, TEMP. The Head module subroutine is used to request which head. Since emitter and detector sides of the incubator are controlled independently, two prefixed (E or D) temperatures will be displayed.

The Growth Decisions module has the function of computing the percentage change of the present channel data from the initial data taken when the Card was first entered into the reading head. These percentage changes are then compared with present threshold values for determination of growth detection. Two commands are available to the operator. STAT can be used anytime and will display a list of abbreviations representing the medias that have gone positive up to that time, if any. FINS will only present a result if the 13 hour incubation period has elapsed. If so, all positive medias and their related antibiotic sensitivities are listed. Included in the list are time elapsed to reach positive threshold and an estimate of enumeration. Logical Card fill errors are also checked and indicated. An example of a logical Card fill error would be enumeration above threshold as determined by MPN theory but the positive control well (same media, approximately 10 times the concentration of organisms) still below threshold. With a diluted sample, enumeration should never be positive when the control well is negative.

The Time History Profile module is activated by the four letter command PLOT. The Monitor first checks that no heads are active (elapsed time greater than zero but less than 13 hours) before allowing a branch to this module. Thus, time history profiles for each channel can be plotted only after all five heads have been inactivated. The space needed for plot data and formats precludes simultaneous data gathering and plotting. Also, the magnetic tape recorder is not a random access unit. Records are only accessed sequentially.

Time history profiles are plotted to a four percent accuracy at half-hour points. This is a compromise between plotting accuracy and plotting time. Headings list incubation head number, channel number associated with each plot symbol, and the related media or antibiotic abbreviations. Tables 3-8 and 3-9 show the position of each well number of the Clinical and Environmental Cards, respectively, as the operator sees them with their related abbreviations. These well numbers are different than those used in the hardware. A table look-up is used to match operator channel with the hardware channel.

These tables also define the one or two character abbreviations used in the MLM and its plots, to the media and antibiotics used in the Card. These abbreviations, based on industry usage, have been selected to prevent duplication and to make maximum use of MLM memory space and limited 16 character alphanumeric display.

The Exit module is used for troubleshooting portions of the MLM. Entrance to this module is from the Monitor by the command EXIT. The Monitor will restrict entrance to this module to times when no head is within the 13 hour incubation period. From this module, the operator may examine memory locations (EX), fill memory locations (FL) or start at any memory location (ST XXXX). PROM memory locations being a read only memory, will be impossible to fill with new memory words.

TABLE 3–8
CLINICAL CARD LAYOUT
WELL NO. AND ABBREVIATIONS

9–1704

1	2	3	4	5	21	22	23	24	25	41	42	43	44	45
EC	FD	SX	NA	AM	PS	AN	NN	GM	CB	AH	K	GM	NA	TE
6	7	8	9	10	26	27	28	29	30	46	47	48	49	50
KE	GM	SX	NA	TE	SR	GM	SX	NA	K	BT	NA	AM	P	ER
11	12	13	14	15	31	32	33	34	35	51	52	53	54	55
CI	FD	SX	NA	TE	GD	FD	ER	TE	AM	YE				+C
16	17	18	19	20	36	37	38	39	40	56	57	58	59	60
PR	K	SX	NA	AM	SA	CF	ER	TE	CC	EN	EN	EN	EN	EN

NOTE: Underlined antibiotics are associated with the media directly to their left.

ABBREVIATIONS AND NAMES

Antibiotics

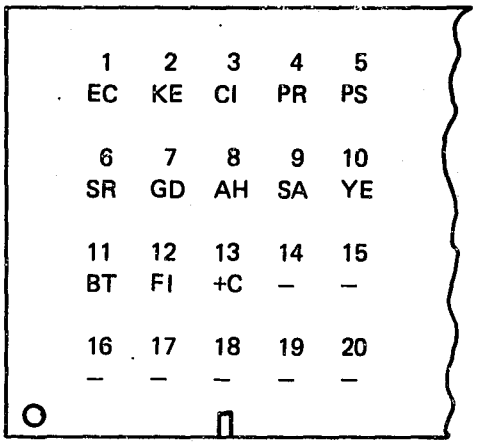
FD = Nitrofurantoin
SX = Trimethoprim-Sulfamethoxazole
NA = Nalidixic Acid
AM = Ampicillin
GM = Gentamicin
K = Kanamycin
ER = Erythromycin
CF = Cephalothin
P = Penicillin
TE = Tetracycline
CC = Clindamycin
CB = Carbenicillin
AN = Amikacin
NN = Tobramycin

Media

EC = E. Coli
KE = Klebsiella-Enterobacter
CI = C. freundii
PR = Proteus
PS = Pseudomonas aeruginosa
SR = Serratia
GD = Group D Enterococcus
SA = Staph aureus
AH = Acinetobacter-Herellea
NA = Group A Beta Strep
BT = Beta Strep Broth
YE = Yeast
+C = Control
EN = Enumeration

TABLE 3-9
ENVIRONMENTAL CARD LAYOUT WELL NO. AND ABBREVIATIONS TYPICAL 3 PLACES

9-1703



ABBREVIATIONS AND NAMES

EC = E. Coli

KE = Klebsiella-Enterobacter

CI = C. freundii

PR = Proteus

PS = Pseudomonas aeruginosa

SR = Serratia

GD = Group D Enterococcus

SA = Staph aureus

AH = Acinetobacter-Herellea

BT = Beta Strep Broth

YE = Yeast

+C = Control

FI = Fungi

4.0 RESULTS

4.1 BIOLOGICAL STUDIES

Major headings discussed under biological results include the following: (1) Selective Media Development; (2) Media Forms and Preparation Methods; (3) Enumeration; (4) Antimicrobial Susceptibility; (5) Storage and Return Capability; (6) Clinical and Seeded Sample Results; and (7) Quality Control and Shelf-Life Studies.

4.1.1 Selective Media Development - Many hundreds of culture media types were screened for their selective ability on microorganisms groups or species in mixed population. The current MLM broths listed in Section 3.1.1 are those media that are able to select for the majority of commonly found medically significant microorganisms. The history and evaluation of these formulations has been previously discussed (7, 8, 9) and will not be repeated here. Significant results were achieved in exploring the many paths that were necessary in arriving at the final formulations. These results representing both successes and failures are discussed in this section.

Chemical Gain and pH Indicators - Although early studies demonstrated MLM capability of detecting microorganism growth in culture media, it was also demonstrated that adding chemical gain indicators which precipitated with slight pH changes gave as much as a seven-fold increased detection speed. Some of the chemical gain indicators used provided an additional bonus of selective inhibition as well. At times, the inhibitory qualities of the chemicals were undesirable in that they inhibited the organism of interest. Early work utilized Congo red as the choice precipitation type indicator signifying pH 6.5 or less. Large scale tests also showed an inhibitory effect for certain organisms, namely: Herellea, Proteus, and Streptococcus. All three organisms are found on the NASA-supplied medically important organisms' list.

Tests with Klebsiella-Enterobacter, Citrobacter, and E. Coli utilized various bile salts compounds as inhibitors. However, in the presence of the appropriate carbohydrates, acid production by these organisms will result in a precipitation of the bile salts. The precipitation of these salts produced an optically detectable reaction and shortened detection time for these organisms.

Early work with Proteus sp indicated urease activity could be optically detected in the presence of magnesium sulfate. Further studies revealed that precipitation was found to occur only when the inoculum size was large and some strains of P. vulgaris would not grow in the medium in less than 48 hours. Thirty different magnesium sulfate indicator modifications were tested with the medium. These modification tests were not successful in resolving the problem and ultimately Brom Thymol blue was substituted and the final Proteus medium relies solely on pH change with no chemical precipitation.

In cases where bile salts could not be used due to toxicity to the organism, a suitable pH indicator was required. Brom Thymol Blue was useful only for a pH shift from neutral to alkaline. As most reactions were due to acid production, an indicator was required that would be optically detectable at 650 nm in a reasonable pH range. A color was needed for detection at this wavelength and no suitable pH indicator satisfied the requirements of color, pH range, nontoxicity, and solubility. The problem was resolved with the discovery that aniline blue when reduced with sodium thioglycollate resulted in a compound that produced a blue color with acid production in the desirable pH range. This indicator was ultimately incorporated into several of the MLM media.

Selective Inhibitors and Enrichment Compounds - The large number of laboratory tests required in selective media development led to the discovery of inhibitors and enrichment compounds previously unreported in literature. In some cases these discoveries simply remained as such due to inability to adapt them to the MLM requirements. Other observations, however, were followed up with large scale testing for validity and incorporated into the MLM media.

It was found that some organisms are tolerant to DP300 - chemical name; 4, 2', 4'-trichloro-2'-hydroxydiphenyl ether (Ciba-Geigy). Reports on minimum inhibitory concentrations of this product did not include tests with some of the organisms studied under this contract. It was found that Serratia sp, and C. freundii tolerated this compound which was ultimately incorporated into formulations.

Tests with uncommonly used carbohydrate sources led to the incorporation of 2-deoxy d Glucose in the Acinetobacter-Herellea medium and plant glycosides in the Serratia medium. These chemicals act as an enrichment or inhibiting agent depending on the organism.

Selective Medium for Shigella Species - Efforts in developing a selective medium for Shigella species were begun almost immediately with slow but continuous improvement throughout the entire period. Some of the approaches explored during this time included various inhibitory chemicals, sole carbon and sole nitrogen sources. Results of some of these tests can be seen in Tables 4-1 through 4-4. Based on results of these screening tests, a tentative selective medium for Shigella species was formulated. The medium utilizes gelysate peptone bile salts, Supplement B, and aniline blue indicator. Various selective inhibitors included phenethylalcohol, and sodium citrate. Shigella organisms rapidly produce a blue color in this medium, which is easily detected by the MLM. The medium was lyophylized for Card use and tested with fecal samples. Although the presence of Shigella was noted by a definite blue color in the broth, fecal samples containing no Shigella gave false positive results due to turbidity of large numbers of normally occurring flora. Since the instrument cannot distinguish between blue color and turbidity, this medium was not used in the MLM system.

Selective Medium for Salmonella Species - The current Salmonella medium containing selenite selects for Salmonella and a few other gram negative organisms. The precipitation of the selenite is indicative of Salmonella sp. However, in the MLM Card environment, turbidity caused by competing gram negatives is not optically distinguishable from selenite precipitation. While this medium has definite value for Salmonella selective isolation, it is not totally inhibitory to competing organisms.

Several other selective formulations were devised with moderate success in clinical testing. These media incorporate Gelysate peptone, d-mannitol, bile salts mixture, and aniline blue indicator. Lithium chloride and tetrathionate are used as inhibitors.

TABLE 4–1
CARBON SOURCES TESTED IN THE DEVELOPMENT OF A SELECTIVE SHIGELLA MEDIUM (EARLY STUDIES)

9–1745

	ORGANISM TESTED			
	SHIGELLA DYSENTERIAE	SALMONELLA PARA TYPHI B	ESCHERICHIA COLI	KLEBSIELLA PNEUMONIAE
CARBON SOURCE				
TWEEN 40	SA	SA	SA	SA
TWEEN 60	SA	SA	SA	SA
VITAMIN B12	±	±	±	±
VITAMIN A	++	++	++	++
CHOLESTEROL	±	±	±	±
THIAMINE HYDROCHLORIDE	±	±	±	±
ALANINE	±	±	±	±
METHIONINE	±	±	±	±
DULCITOL	±	+, SA	±	±
HEPARIN	–	–	–	–
INULIN	+	+, SA	+	+
SODIUM BUTYRATE	∓	∓	∓	∓
SODIUM PROPIONATE	∓	∓	∓	∓
GASTRIC MUCIN	∓	∓	∓	∓
6 DEOXYMANNOSE	∓	∓	±±	±±
SODIUM PYRUVATE	++	+	++	++
β GLOBULIN FACTOR 3	–	–	–	–
β GLOBULIN FACTOR 4	–	–	–	–
CARBOXYLASE	–	–	–	–
α GLOBULIN	–	–	–	–
MELITIN	–	–	–	–
MUCIN	–	–	–	–
BETAINE	–	–	–	–
α KETOGLUTARIC ACID	++	SA ++	++	++
NICOTINIC ACID	–	–	–	–
SERINE	+	+	+	++
SUCCINIC ACID	–	–	–	–
CHOLESTEROL PALMITATE	–	±	±	±
ASCORBIC ACID	–	+	+	+
CARDIOLIPIN	±	±	+	±
TURANOSE	–			
XYLAN	–			
PALATINOSE	–			
GIBBERELLIC ACID	±	+		
ESCULIN	+	++		
CAFFEINE	+	+, SA		
8 CHLOROXANTHINE	+	+		
L THREONINE	+	+		
DL HISTIDINE	+	+		
ETHYL INDOLE ACETATE	+	+		
6 BROMOPURINE	+	+		
BROMELAIN	+	+		
DL PANTOYL LACTONE	–	–		
VIOLURIC ACID	∓	±		
L-TRYPTOPHAN	–	+		
HYDROLYZED INULIN	–	+		+

++; GROWTH
++; HEAVY GROWTH
±; WEAK POSITIVE
–; NEGATIVE GROWTH
SA; SLIGHT ACID

TABLE 4–2

CARBON SOURCES TESTED IN THE DEVELOPMENT OF A SELECTIVE SHIGELLA MEDIUM 9–1744

	ORGANISM TESTED			
	SHIGELLA DYSENTERIAE	SALMONELLA PARA TYPHI B	ESCHERICHIA COLI	KLEBSIELLA PNEUMONIAE
HYDROLYZED ARABINOSE	–	+		+
HYDROLYZED XYLOSE	–	+		+
CALF SERUM	±	±	++	
ROYAL JELLY	–	–	+	
CREATINE	–	–	+	
GLOBULIN IV–1	–	–	–	
SUPPLEMENT XV FACTORS	–	++	++	
GLUTAMINE	–	++	++	
ARGININE	–	–	±	
DL PHENYLALANINE	–	–	–	
THREONINE	–	–	–	
RIBOFLAVIN	–	–	–	
VIOLURIC ACID	–	–	–	
GLYCINE	–	–	+	
LECITHIN SOY	+	+		
SODIUM CAPROATE	–	–		
SODIUM VALERATE	–	+		
SODIUM FUMARATE	–	+		
SAPONIN	+	+		
IRON DNA	–	+		
CELLOBIOSE	–	–	+	
TYROSINE	–	–	–	

+ ; GROWTH
– ; NEGATIVE GROWTH
++ ; HEAVY GROWTH
± ; WEAK POSITIVE

Selective Medium for Beta Streptococci - Three formulations have been extensively studied in efforts to arrive at a medium compatible with the MLM Card concept. Formulations are listed in Section 3.1.1, Selective Media Development. These formulations have worked very well in liquid broth. Attempts to convert the formulations to lyophilized form, however, have met with limited success. Streptococcus medium #1 is a general purpose streptococcus broth allowing growth of all streptococcus groups. The addition of red blood cells provides a visual indicator for the beta hemolytic streptococci. Efforts to preserve the rbc's for the lyophilization process failed, resulting in a useless formulation for future Card studies.

Streptococcus Medium #2 had limited success in subsequent Card adoption. The fibrinolytic action of the Group A beta streptococci could be demonstrated in liquid fill tests with the MLM. Although the medium continued to perform following liquid

TABLE 4–3
INHIBITORY CHEMICALS SCREENED IN THE SHIGELLA BASE WHICH CONTAINS PHENETHYL ALCOHOL

9–1743

CHEMICAL	Organisms Tested											
	CONCENTRATION GRAMS PERCENT	SHIGELLA DYSENTERIAE	SHIGELLA BOYDII	SHIGELLA SONNEI	SHIGELLA FLEXNERI	ALCALESCENS DISPAR	SALMONELLA SPECIES	KLEBSIELLA PNEUMONIAE	ENTEROBACTER SPECIES	CITROBACTER SPECIES	SERRATIA MARCESCENS	PROTEUS SPECIES
BASE PHENETHYL ALCOHOL	0.15	+	±	+	+	+	±	±	–	+	–	+
BASE PHENETHYL ALCOHOL	0.2	±	–	+	+	+	–	–	–	±	–	+
LITHIUM CHLORIDE	0.2	–	–	+	+	+	+	–	–	±	–	±
BENZIDINE	0.05	–	–	+	–	+	+	–	–	+	–	+
POTASSIUM IODIDE	0.5	+	+	+	+	+	+	–	–	+	–	+
POTASSIUM IODIDE	2	+	–	+	–	+	+	–	–	+	–	+
POTASSIUM FLUORIDE	0.1	+	–	+	+	+	+	–	–	+	–	+
POTASSIUM FLUORIDE	0.3	±	–	+	+	±	+	–	–	+	–	+
SODIUM PERIODATE	0.05	–	–	+	–	+	–	–	–	–	–	–
LITHIUM NITRATE	0.2	+	–	+	+	+	+	–	–	+	–	+
BROMO ANILINE	15 mg	+	+	+	+	+	–	–	–	–	–	+
BROMO ANILINE	30 mg	–	±	+	+	+	–	–	–	+	–	+
SODIUM PERIODATE	0.01	+	–	+	+	+	+	–	–	+	–	+
SODIUM CITRATE	0.05	+	±	+	+	+	–	–	–	±	–	+
SODIUM CITRATE	0.25	+	–	+	+	+	–	–	–	–	–	+
SODIUM CITRATE	0.5	+	–	+	+	+	–	–	–	–	–	+
SODIUM CITRATE	1	–	–	–	–	–	–	–	–	–	–	+

+ ; POSITIVE GROWTH
– ; NEGATIVE GROWTH
± ; WEAK POSITIVE
U ; UNDETERMINED, LOW SOLUBILITY

BASE MEDIUM
1% BILE SALTS MIXTURE
5% GELYSATE PEPTONE
1% SUPPLEMENT B
0.15% PHENETHYL ALCOHOL
7.2 FINAL pH

MICROBIAL LOAD MONITOR

9–1742

TABLE 4–4

INHIBITORY CHEMICALS SCREENED IN THE DEVELOPMENT OF A SHIGELLA MEDIUM

CLINICAL	CONCENTRATION GRAMS PERCENT	ORGANISM TESTED: S. DYSENTERIAE	S. SONNEI	S. FLEXNERI	SALMONELLA COLUMBIENSIS	PSEUDOMONAS AERUGINOSA	E. COLI	KLEBSIELLA SPECIES	SERRATIA MARCESCENS	PROTEUS SPECIES
BRUCINE SULFATE	1	+	+	+	+	+	+	+	+	+|
THIOSEMICARBAZIDE	0.5	–	–	+	+	–	+	+	–	–
β (2 FURYL) ACRYLIC ACID	0.5	+	+	+	+	+	+	+	+	+
2, 4, 5, TRICHLOROACETIC ACID	0.5	+	+	+	+	+	+	+	+	+|
RHODANINE	2	–	–	–	–	–	+	–	–	–
BROMINE PYRIDIUM	0.4	–	–	+|	–	–	+|	–	–	–
2, 4, 5 TRICHLOROPHENOXY ACETIC ACID	0.5	+	+|	+	+	+	+	+	+	+
GERMANIUM DIOXIDE	0.05	–	+	+	+	–	+	+	+	+
MERTHIOLATE	0.4	–	+	–|	+	+	+	+	+|	+
CESIUM CHLORIDE	1	–	–|	+	+	–|	+	+	+|	–
CERRIC AMMONIUM NITRATE	U	–	–|	+	+	+	+	+	+|	–
SILVER DIETHLY DITHIOCARBONATE	U	+	+	+	+	+	+	+	+|	–|
ISONIAZIDE	1	–	+	+	–	–	+	+	+|	+|
PHENYLSEMICARBAZIDE	0.025	+	+|	+	+	+	+	+	+	–
PHENYLUREA	U	–	–	+|	–	–	+	+	–	–
PHENYL ACETATE	U	–	+|	+	+	–|	+	+	+	–
IODINE	0.5	+	–	+	+	+	+	–	–	+
IODINE	1	–	–	–	–	+	–	–	–	+
IODOANLINE	U	–	–	+	–	–|	+	+	+	+
ANTIMONY TRICHLORIDE	U	–	+	+	–	+|	–	–	–	+|
CUPRIC SULFATE	0.01	+	+	+	+	+	+	+	+	+
COBALT NITRATE	0.05	–	–|	–	+	+	+	+	+	–
STRONTIUM NITRATE	1	–	–	+	+	–	+	–	–	–
BISMUTH SULFATE	U	–	+	+	+	+	+	+	+	+
RUBIDIUM ACETATE	0.5	–	+|	+	+	+|	+	+	–	–
RUBIDIUM ACETATE	1.5	–	–	+	–	–	+	–	–	–
RUBIDIUM ACETATE	2	–	–	+	–	–	–	–	–	–
STANNOUS FLUORIDE	U	+	+|	+	+|	–	+|	+	+	+
SODIUM CHROMATE	0.05	+|	+	+	+	+	+|	+	+	+
QUINOLINE	0.02	–	–	–	+	–|	+	+	+	+
LITHIUM CHLORIDE	1	–	–	–	–	+|	–	–	–	+|
THIMEROSAL	0.01	–	–	–	–	–|	–	–	–	–
PHENETHYL ALCOHOL	0.2	–	+	+	+	–	–	–	–	–
PHENETHYL ALCOHOL	0.25	–	–	+	–	–	–	–	–	–
PHENYLENEDIAMINE	0.05	+	+	+	+	+|	+	+	+	+
1, 3 DIPHENYL GUANIDINE	0.1	+	+	+	+	+|	+	+	–	–
GUANIDINE NITRATE	1	–	–	–	+|	+	–	–	–	–
XANTHINE	U	+	+	+	+	+	+	+	+	+
SODIUM PERBORATE	1	–	–	–	–	–	–	–	–	–
QUINOLINIC ACID	1	+	+	+	+	+	+	+	+	+
M–DINITROBENZENE	0.1	–	–	–	–	–	–	–	–	–
2, 4 DINITROANISOLE	U	+	+	+	+	+	+	+	+	+
2, 4, 5 TRICHLOROPHENOXY PROPIONIC ACID	0.1	+	+	+	+	+	+	+	+	+

+; POSITIVE GROWTH
–; NEGATIVE GROWTH
+–, –+; WEAK POSITIVE
U; UNDETERMINED, PPT FORMED, OR LOW SOLUBILITY

BASE MEDIUM
3% BILE SALTS MIXTURE
5% GELYSATE PEPTONE
1% SUPPLEMENT B
7.5 FINAL PH

fill tests with the MLM. Although the medium continued to perform following lyophilization, sensitivity was reduced and MLM detection time was not within the required time frame of 13 hours.

Formulation #3 represents the most successful effort to date and is incorporated into the current MLM Cards. This medium consists of an enriched base to which various inhibitors have been added to enhance selectivity. The broth has a red color upon rehydration. A positive is indicated by a change in color to purple. This formulation has limitations for freeze dried application, however. Large numbers of competing organisms such as found in fecal specimens will overwhelm the broth resulting in a false positive reaction. If the broth is applied to throat or sputum cultures it will perform with an accuracy of 95%.

4.1.2 Media Forms and Preparation Methods - Several media forms and preparation methods proved to be unsuccessful in early testing. These included pellets, saturated porous discs and platicized media wafer.

The pellet preparation was an extremely tedious process and resulted in non-uniform media constituents. Rehydration was slow or incomplete, leaving many bubbles in the well viewing area. The saturated discs required a concentration procedure of the media constituents and had the additional disadvantage of blocking the viewing wells.

The plasticized media wafers were easy to prepare and could be adapted to a large volume automated approach. However, large scale testing demonstrated that media efficiency was altered and that the plasticizers remained in solution interfering with optical detection.

Freeze dried preparation resulted in the most reliable test results. Early attempts to incorporate this method of media preparation were met with limited success. As the contract progressed, however, many of the technological problems encountered in high volume preparation were resolved. The current MLM Card utilizes lyophilized media. Rehydration is nearly complete within 5 minutes of sample introduction. Uniformity and consistency of results have been demonstrated from lot to lot and bubble interference is minimal.

Table 4-5 summarizes the various media preparation techniques and notes advantages and disadvantages of each.

Tables 4-6 and 4-7 list results obtained in early tests utilizing both liquid and plasticized film media. Table 4-8 summarizes some early clinical results utilizing freeze dried formulations.

TABLE 4-5
MEDIA LOADING TECHNIQUES COMPARISON
9-1741

TECHNIQUE	ADVANTAGE	DISADVANTAGE
1. FREEZE DRIED MEDIA		
a. POWDERED	MODERATE PREPARATION COMPLEXITY	DIFFICULT CARD LOADING PROCESS; WASHES OUT OF VIEWING WELL
b. PELLETS	IDEAL PHYSICAL CHARACTERISTICS, OPTIMUM REHYDRATION RATE	COMPLEX PREPARATION; DIFFICULT HANDLING AND CARD LOADING PROCESS
c. IN SITU FREEZE DRIED	MINIMAL PREPARATION COMPLEXITY	DRYING TECHNIQUE CRITICAL SPLATTER MAY CONTAMINATE ADJACENT WELLS.
2. LIQUID MEDIA		
a. IN SITU DRIED	EASE OF CARD LOADING, EASE OF AUTOMATION	REHYDRATES RAPIDLY, MAY WASH FROM VIEWING WELL INTO OVERFLOW WELL
b. SATURATED DRIED POROUS DISC	EASE OF CARD LOADING AND STORAGE OF MEDIA	DISC CANNOT BE PLACED IN VIEWING WELL, DIFFICULT TO REHYDRATE
3. PLASTICIZED MEDIA		
a. WAFER	EASE OF PREPARATION, STORAGE AND AUTOMATION	PLASTICIZERS REMAIN IN SOLUTION AND ALTER MEDIA EFFICIENCY

4.1.3 Enumeration Studies

Disc Filtration Method - One of the basic tasks of the MLM is a determination of relative numbers of detected organisms. Background testing with hand-loaded cassettes proved the feasibility of the asbestos filter system. Preliminary data collected under previous contracts had suggested that true filtration was not involved and that serial dilution was due to surface absorption of microbes on asbestos fibers. Experiments such as reported in Table 4-9 proved that the serial dilution in the plastic cassettes was possible, and indicated that time required for filling was a factor in successful one log serial dilution. In these studies,

TABLE 4–6
CLINICAL SAMPLES RESULTS WITH LIQUID MLM MEDIA

9–1739

MEDIUM	136 URINE	38 SPUTUM		DETECTED OTHER ORGANISMS (FALSE POSITIVES)		TIMES EXCEEDED STANDARD TESTS
	POSITIVES (SELECTIVE MEDIUM)	FAILED TO DETECT (FALSE NEGATIVES)				
COLIFORM	74	1/74	1.3%	1/100	1%	6
PSEUDOMONAS	28	0/20	0%	8/146	5%	4
PROTEUS	29	1/27	3.7%	2/145	1.3%	0
CANDIDA	6	1/4	25%	2/168	1.1%	0
SALMONELLA	13*	1/7	14.2%	6/123	4.8%	0
MIMA-HERELLEA	6*	3/6	50%	1/131	0.7%	0
N. MENINGITIDIS	3	0/0	0%	2/38	5.3%	0
D. PNEUMONIAE	8	3/7	43%	3/32	9.4%	1
FUNGI	0	0/0	0%	2/136**	1.3%	0
S. AUREUS	3	0/3	0%	0/171	0%	0

*SEEDED SPECIMEN
**YEAST DETECTED

serially diluted inocula were removed from the detection wells and plated by standard methods to determine numbers present. Similar tests presented in Tables 4-10 and 4-11 confirmed these facts. Mixed culture studies as presented in Table 4-12 proved that pure cultures were not required for serial dilution.

For high volume MLM tests, over 2,500 plastic cassettes had to be loaded with filtration beds. Analysis of the high volume data reveals that for the most part the filtration system was faulty, and rarely worked as it should. In many cases, the filters were so tightly packed that a proper vacuum was not achieved for proper cassette filling. In other cases, the packing was uneven with some wells in the series having twice as much filter material as they should have contained. Another problem area was that of MLM system sensitivity. The filter system's effectiveness in serially reducing the numbers of organisms present in the original sample resulted in extended (>14 hrs) detection times for some Pseudomonas at 5×10^4 to 1×10^5 range.

TABLE 4–7
CLINICAL SAMPLES RESULTS WITH PLASTICIZED MLM MEDIA

9–1740

MEDIUM	236 URINE	65 SPUTUM				TIMES EXCEEDED STANDARD TESTS
	POSITIVES (SELECTIVE MEDIUM)	FAILED TO DETECT (FALSE NEGATIVES)		DETECTED OTHER ORGANISMS (FALSE POSITIVES)		
COLIFORM	142	5/141	3.5%	1/159	0.6%	4
PSEUDOMONAS	22	8/13	61%	9/282	3.2%	2
PROTEUS	42	8/40	20%	2/254	0.8%	1
CANDIDA	20	0/12	0%	8/281	2.8%	2
SALMONELLA	8*	12/14	85%	6/222	2.7%	0
MIMA-HERELLEA	2*	8/9	89%	1/227	0.4%	0
N. MENINGITIDIS	2	1/1*	100%	2/65	3.0%	0
D. PNEUMONIAE	7	7/7	100%	6/65	9.2%	1
FUNGI	3	0/0	0%	3/236**	1.2%	0

*SEEDED SPECIMEN
**YEAST DETECTED

Current MLM Enumeration - Data establishing validity and reliability of the current MLM enumeration scheme were gathered over a 2-year period and represent more than 3000 clinical samples. Table 4-13 summarizes enumeration results from 1987 clinical urine cultures showing either negative results or one strain of organism. Table 4-14 lists results of 2096 mixed culture clinical urines. These data represent tests performed prior to MLM completion and is a summary of AMS results. The AMS utilizes the same sample dilution, enumeration media, and total number of enumeration wells. These data were useful in early establishment of this enumeration method. As indicated by the data, the lowest correlation to standard enumeration results was obtained for yeasts with a percent reliability of 76.3. False negatives contributed heavily to the overall correlation figure. False negative here is defined as the times that yeast were present in the sample at $\geq 10^5$/ml but were enumerated by the AMS to be $<10^5$/ml. Two possible reasons for this disappointing correlation are the relative size of yeast cells in comparison to bacteria and increased detection time with these organisms.

TABLE 4–8

CLINICAL SAMPLES RESULTS WITH MLM FREEZE DRIED MEDIA

9–1738

MEDIUM	SAMPLES	POSITIVES (SELECTIVE MEDIUM)	FAILED TO DETECT (FALSE NEGATIVES)	DETECTED OTHER ORGANISMS (FALSE POSITIVES)	TIMES EXCEEDED STANDARD TESTS
COLIFORM	413	104	1/102 0.9%	0/309 0%	2
PSEUDOMONAS	413	22	0/22 0%	1/391 0.25%	4
PROTEUS	413	43	3/43 7%	23/370 6.2% 0/271 AFTER ADDITION OF COLISTIN 0%	3
STAPHYLOCOCCUS AUREUS	413	21	1/16 6.2%	1/392 0.25%	5
CANDIDA ALBICANS	413	8	1/7 14%	0/406 0%	1
SALMONELLA	322	0	1*	0/321 0%	0
ASPERGILLUS	322	0	0 (NONE FOUND)	2/322** 0.6%	0
MIMA–HERELLEA	180	0	0 (NONE FOUND)	0/180 0%	0
N. MENINGITIDIS	91	0	0 (NONE FOUND)	0/91 0%	0
BETA STREPTOCOCCUS	211	2	0/2 0%	0/269 0%	0
KLEBSELLA/ ENTEROBACTER	271	13	1/13 7/7%	2/258 0.8%	0

*S. TYPHIMURIUM
**DETECTED CANDIDA ALBICANS

197 URINES
140 THROAT/SPUTUM
47 WOUNDS
29 FECES

TABLE 4–9
DILUTION ABILITY OF ASBESTOS IS DIRECTLY DEPENDENT ON THE ABSORPTION RATE OR FILLING TIME OF CASSETTE

Growth Chamber Assay E. Coli/ml

9–1736

STARTING CELL SUSPENSION	TIME OF CASSETTE FILLING	AMOUNT OF ABESTOS	TRIAL NO.	GROWTH CHAMBER II	GROWTH CHAMBER III	GROWTH CHAMBER IV	APPROXIMATE AVE. LOG REDUCTION
5×10^7	4 SEC	0.001g	1	8×10^6	2×10^6	4×10^5	0.7
5×10^7	4 SEC	0.001g	2	1×10^7	7×10^6	1×10^6	0.5
5×10^7	4 SEC	0.001g	3	9×10^6	4×10^6	8×10^5	0.55
5×10^7	4 SEC	0.001g	4	2×10^7	9×10^6	6×10^6	0.3
5×10^7	7 SEC	0.001g	5	1×10^7	6×10^6	2×10^6	0.45
5×10^7	7 SEC	0.001g	6	8×10^6	2×10^6	7.5×10^5	0.52
5×10^7	8 SEC	0.001g	7	2.5×10^7	8×10^6	1×10^6	0.6
3×10^7	8 SEC	0.001g	8	9×10^6	2×10^6	3×10^5	0.8
3×10^7	10 SEC	0.001g	9	7×10^6	1×10^6	8×10^4	0.9
3×10^7	12 SEC	0.001g	10	1×10^7	5×10^6	6×10^4	1.25
3×10^7	12 SEC	0.001g	11	7.5×10^6	9×10^5	1×10^5	0.82
3×10^7	15 SEC	0.001g	12	8×10^6	3×10^5	5×10^4	1.15
3×10^7	15 SEC	0.001g	13	9.5×10^6	1.5×10^5	2×10^4	1.3
2.5×10^7	15 SEC	0.001g	14	5×10^6	1.3×10^5	1.5×10^4	1.2
2.5×10^7	20 SEC	0.001g	15	7×10^6	5×10^5	6×10^4	1
2.5×10^7	25 SEC	0.001g	16	1×10^6	2×10^5	1.5×10^4	1
2.5×10^7	30 SEC	0.001g	17	3×10^6	5×10^5	2×10^4	1
4×10^7	1 MIN	0.001g	18	8×10^6	1.8×10^5	1.3×10^3	1.8
1×10^7	1 MIN	0.001g	19	3×10^6	5×10^4	7×10^2	1.8
4×10^7	2 MIN	0.001g	20	3×10^4	1×10^2	0	2.5
4×10^7	2.5 MIN	0.001g	21	7×10^3	0	0	3
4×10^7	3 MIN	0.001g	22	1×10^2	0	0	4

The data in Table 4-13 and 4-14 were based on an AMS threshold of 10% attenuation by three or more of the five enumeration wells within a 13-hour time frame to establish an original inoculum number of >70,000 cfu/ml.

Tests were later conducted on the MLM to validate these earlier results. Results of these tests showed 97% correlation to the standard dilution - plate method of enumeration and are included in reliability results (4.3.1).

TABLE 4–10

CORRELATION OF CASSETTE FLUID FILLING TIME WITH SERIAL LOG REDUCTION IN MICROBIAL INOCULUM

9–1737

TEST ORGANISM	RANGE OF SECONDS REQUIRED TO FILL CASSETTE	EXAMPLES OF ACTUAL MICROBIAL COUNT IN CASSETTE GROWTH WELLS AFTER SUCCESSIVE FILTRATION			AVERAGE TIME OF FILL RESULTING IN 1 LOG SERIAL REDUCTION (IN SECONDS)
		WELL 2	WELL 3	WELL 4	
STAPHYLOCOCCUS AUREUS	4–5	5×10^5	7×10^4	3×10^3	4.5
β HEMOLYTIC STREPTOCOCCUS	3–5	5×10^5	6×10^4	2×10^3	4
PROTEUS MORGANII	15–20	6×10^4	5.0×10^3	1×10^2	17.5
PSEUDOMONAS AERUGINOSA	10–15	6×10^4	7.5×10^3	9×10^2	12.5
KLEBSIELLA PNEUMONIAE	12–18	4×10^4	1.3×10^3	4×10^2	15
ESCHERICHIA COLI	15–25	7×10^6	5×10^5	6×10^4	20
CANDIDA ALBICANS	8–10	7×10^5	4×10^4	3.5×10^3	9
HERELLEA VAGINICOLA	10–15	2×10^5	5×10^4	4×10^3	12.5
DIPLOCOCCUS PNEUMONIAE*	3–4	3×10^5	5×10^4	8×10^3	3.5
STREPTOCOCCUS FAECALIS	10–15	5×10^5	7×10^4	9×10^3	12.5
SALMONELLA TYPHOSA	18–20	3×10^5	5×10^4	6×10^3	19
MIMA POLYMORPHA	8–12	6×10^5	3×10^4	4.3×10^3	10

*ENCAPSULATED STRAIN, ROUGH STRAINS REQUIRED 2 SECONDS TO PRODUCE 1 LOG REDUCTION

Clinically significant bacteriuria has been generally reported at $\geq$ 100,000 cfu/ml (1×10^5). For this reason, MLM testing was performed to establish best correlation of the MLM Clinical Card to standard method of enumeration. Examination of MLM test data determined that optimum threshold for this instrument to be 15% and that best correlation results utilized five of five enumeration wells to indicate positive to correlate to $\geq 10^5$ organisms/ml.

Overall reliability of the AMS-MLM enumeration method is approximately 94% to standard dilution and plating methods.

TABLE 4–11
RELIABILITY OF AN ASBESTOS FILTRATION SYSTEM
Growth Chamber Assay E. Coli/ml

9–1735

STARTING CELL SUSPENSION	TIME OF CASSETTE FILLING (SEC)	AMOUNT OF ABESTOS	TRIAL NO.	GROWTH CHAMBER II	GROWTH CHAMBER III	GROWTH CHAMBER IV	APPROXIMATE AVE. LOG REDUCTION
3×10^7	3	0.00075g	1	8×10^6	3×10^6	5×10^5	0.65
3×10^7	4	0.00075g	2	6×10^6	2.5×10^6	3×10^5	0.65
3×10^7	4	0.00075g	3	7.5×10^5	1×10^6	9×10^4	0.85
3×10^7	3	0.00075g	4	9×10^6	5×10^6	9×10^5	0.5
3×10^7	3	0.00075g	5	1.5×10^7	7×10^6	1×10^6	0.5
3×10^7	4	0.00075g	6	4×10^6	9×10^5	5×10^5	0.45
3×10^7	3	0.00075g	7	8×10^6	4.5×10^6	1×10^6	0.35
5×10^7	3	0.00075g	8	1×10^7	6×10^6	9.5×10^5	0.6
5×10^7	2	0.00075g	9	3×10^7	9×10^6	8×10^6	0.25
5×10^7	3	0.00075g	10	2×10^7	9×10^6	2×10^6	0.5
5×10^7	2	0.00075g	11	4×10^7	1×10^7	8×10^6	0.3
5×10^7	2	0.00075g	12	3.5×10^7	9.5×10^6	8×10^6	0.27
5×10^7	3	0.00075g	13	1×10^7	6×10^6	2×10^6	0.45
2×10^7	3	0.00075g	14	9×10^6	5×10^6	9.5×10^5	0.47
2×10^7	4	0.00075g	15	7.5×10^6	2×10^6	7×10^5	0.52
2×10^7	3	0.00075g	16	9×10^6	6×10^6	2×10^6	0.35
2×10^7	3	0.00075g	17	8×10^6	5×10^6	1×10^6	0.4
3×10^7	2	0.00075g	18	2.5×10^7	9×10^6	8×10^6	0.22
1×10^7	4	0.00075g	19	6×10^6	1×10^6	5.5×10^5	0.47
1×10^7	5	0.00075g	20	5.5×10^6	1×10^6	6×10^5	0.47
1×10^7	4	0.00075g	21	7.5×10^6	2×10^6	8×10^5	0.47
5×10^7	3	0.00075g	22	1×10^7	7×10^6	2×10^6	0.4
5×10^7	3	0.00075g	23	2.5×10^7	8×10^6	4×10^6	0.42
5×10^7	2	0.00075g	24	2×10^7	9×10^6	7×10^6	0.25
5×10^7	3	0.00075g	25	3×10^7	8×10^6	5.5×10^6	0.42

4.1.4 Antimicrobial Susceptibility Test Results - Early contract work centered around selecting those antimicrobials currently in use by local hospitals and establishing a single optimum concentration for each drug in each selective formulation. The lyophilized media-drug combinations were then tested in the MLM and results were compared to standard Kirby-Bauer disc diffusion method. Early test results presented in Table 4-15 established the feasibility of the selective media approach to antimicrobial susceptibility testing.

TABLE 4–12
SERIAL LOG DILUTION IN FILTER CASSETTES WITH MIXED CULTURES

9–1734

TEST ORGANISM	STARTING CELL SUSPENSION	SECONDS REQUIRED TO FILL CASSETTE	MICROBIAL COUNT IN CASSETE GROWTH WELLS AFTER FILTRATION		
			WELL 1	WELL 2	WELL 3
ESCHERICHIA COLI	4×10^6/ml	14	5×10^4	3×10^2	$< 10^1$
ESCHERICHIA COLI PLUS STAPHYLOCOCCUS AUREUS	4×10^6/ml 3×10^4/ml	20	3×10^4	2×10^2	$< 10^1$
ESCHERICHIA COLI PLUS STAPHYLOCOCCUS AUREUS	4×10^6/ml 7×10^6/ml	13	8×10^4	1.3×10^3	$< 10^2$
PSEUDOMONAS AERUGINOSA	5×10^6/ml	15	5×10^5	3×10^4	5×10^2
PSEUDOMONAS AERUGINOSA PLUS STAPHYLOCOCCUS AUREUS	5×10^6/ml 3×10^4/ml	10	1×10^5	2.5×10^4	7×10^2
PSEUDOMONAS AERUGINOSA PLUS STAPHYLOCOCCUS AUREUS	5×10^6/ml 7×10^6/ml	10	4×10^5	1.5×10^4	*

*SAMPLE ERROR

The enrichment-selective media were generally well-suited for the performance of antimicrobial susceptibility determinations. However, minor modifications in the formulations of the specific media were required to accommodate the addition of antibiotics and chemotherapeutic agents. These selective-enrichment media of the MLM Card suitable for antimicrobial susceptibility testing are present as a series of 5 wells per medium. For example, wells 1 through 5 of the MLM Clinical Card contain the selective formulation for identification (growth) of E. coli. Well #1 is designated the positive or control well and contains the selective broth with no antimicrobials. The remaining four wells contain E. coli selective medium plus a designated antimicrobial agent. Bacteria are reported as "Resistant" when detection in a specific well exceeds preset thresholds. Conversely, a culture is reported as "susceptible" when there is either no microbial activity in the specific antibiotic well or when the activity is insufficient to exceed preset thresholds. For any selective medium antimicrobial series to be reported the control well (containing no antimicrobial) must exceed the preset threshold. No Kirby-Bauer equivalent of "Intermediate" or "Indeterminate" category is registered by the MLM. Figures 4-1 through 4-4 present data as typically recorded by the MLM.

TABLE 4–13
ENUMERATION STUDIES AMS METHOD VS STANDARD DILUTION & COUNT SINGLE STRAIN – 1987 CLINICAL URINES

9–1642

Organism	Number Positive	Number Negative	True Positive	True Negative	False Positive	False Negative	% (+)	% (–)	% Reliability
Pseudomonas	17	4	17	3	1	0	100.0	75.0	95.2
Proteus sp	32	26	32	21	5	0	100.0	80.8	91.4
C. freundii	0	0	0	0	0	0	0	0	0
Serratia sp.	1	0	1	0	0	0	100.0	0	100.0
E. coli	238	95	233	72	23	5	97.9	75.8	91.6
Klebsiella – Enterobacter	46	11	46	9	2	0	100.0	81.8	96.5
Yeast	23	15	17	12	3	6	73.9	80.0	76.3
Group D enterococcus	33	54	25	51	3	8	75.8	94.4	87.4
S. aureus	4	2	4	2	0	0	100.0	100.0	100.0
Other	16	43	11	39	4	5	68.7	90.7	84.7
No growth	–	1327	0	1289	30	0	0.0	97.1	97.1
Overall									94.8

$$\%(+) = \frac{T(+)}{T(+) + F(-)} \times 100$$

$$\%(-) = \frac{T(-)}{T(-) + F(+)} \times 100$$

$$\%\ REL. = \frac{T(+) + T(-)}{[T(+) + T(-) + F(+) + F(-)]} \times 100$$

As the present contract progressed, some media were "fine-tuned" in efforts to continually upgrade and ultimately optimize media performance and thereby decrease detection time. New antimicrobials such as amikacin, tobramycin, and carbenicillin were tested for performance in Pseudomonas selective medium. For these reasons, additional studies were performed and are presented in Table 4-16. The four selective media and respective drug combinations presented on this table were those studied most extensively as a result of developmental progress achieved early in the contract. The remaining selective media developments occurred during the progression of contract work, and in some cases (Beta streptococcus medium) have been only recently optimized. Developmental results of antimicrobial susceptibility tests with these formulations are summarized in Table 4-17.

TABLE 4–14

ENUMERATION STUDIES AMS METHOD VS STANDARD DILUTION AND COUNT MIXED CULTURES (2096 Clinical Urines)

9–1656

Organism	Number Positive	Number Negative	True Positive	True Negative	False Positive	False Negative	% (+)	% (–)
Pseudomonas	19	18	19	7	1	0	100.0	87.5
Proteus	33	36	33	29	7	0	100.0	80.6
C. freundi	0	0	0	0	0	0	0	0
Serratia sp	1	0	1	0	0	0	100.0	0
E. coli	249	123	244	92	32	5	98.0	74.0
Klebsiella-Enterobacter	53	13	53	9	4	0	100.0	69.2
Yeast	25	16	19	13	3	6	76.0	81.2
Group D enterococcus	42	72	33	62	10	9	78.6	86.1
S. aureus	4	3	4	3	3	0	100.0	100.0
Others	19	53	14	47	6	5	73.7	88.7
No Growth	–	1327	0	1289	38	0	0	97.1
Overall								94.0

$$\%\,(+) \quad \frac{T\,(+)}{T\,(+) + F\,(-)} \times 100$$

$$\%\,(-) = \frac{T\,(-)}{T\,(-) + F\,(+)} \times 100$$

Overall MLM performance and reliability tests are discussed and results are presented in Section 4.3, Reliability Tests.

4.1.5 Storage and Return Capability - To determine the optimum treatment of the MLM detected microorganisms for maximum survival after storage and return to earth, several procedures were examined as outlined in Section 3.1.6. Results of these tests are presented in this section.

Freezing Technique versus Storage Test Results - Results of the quick freeze tests are found in Table 4-18. Of the eleven organisms tested, only three failed to yield viable cultures after the 130-day storage period.

Table 4-19 shows results of a slow freezing method in which the temperature was lowered 1°C every minute until -40°C was reached. This same method was used in two other slow freeze experiments shown in Tables 4-20 and 4-21, except that

TABLE 4–15
EARLY RESULTS OF ANTIBIOTIC DETECTION TRIALS IN MLM

9–1718

SPECIMEN	MEDIUM	ORGANISM	ANTIBIOTIC AND MEDIUM CONCENTRATION (MCG/ML)	MLM RESULTS	KIRBY-BAUER STANDARD RESULTS
URINE	DIPLOCOCCUS PNEUMONIAE	D. PNEUMONIAE NO. 1	PENICILLIN 10	S*	S
			TETRACYCLINE 10	S	S
			ERYTHROMYCIN 5	S	S
			CLINDAMYCIN 2	S	S
			OXACILLIN 1	S	S
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	DIPLOCOCCUS PNEUMONIAE	D. PNEUMONIAE NO. 4	PENICILLIN 10	S	S
			TETRACYCLINE 10	S	S
			ERYTHROMYCIN 5	S	S
			CLINDAMYCIN 2	S	S
			OXACILLIN 1	S	S
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	NEISSERIA MENINGITIDIS	N. MENINGITIDIS	PENICILLIN 10	S	S
			TETRACYCLINE 10	S	S
			ERYTHROMYCIN 5	S	S
			CLINDAMYCIN 2	R**	R
			OXA CILLIN 1	R	R
		POSITIVE CONTROL	NONE	GROWTH	
URINE	PROTEUS	P. MIRABILIS	NITROFURANTOIN 5	R	R
			TETRACYCLINE 10	R	R
			NALIDIXIC ACID	R	R
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	PROTEUS	P. MORGANII	NITROFURANTOIN 5	R	R
			TETRACYCLINE 10	R	I
			NALIDIXIC ACID	R	S
		POSITIVE CONTROL	NONE	GROWTH	
FECES	PROTEUS	P. MIRABILIS NO. 2	NITROFURANTOIN 5	R	R
			TETRACYCLINE 10	R	R
			NALIDIXIC ACID	R	I
		POSITIVE CONTROL	NONE	GROWTH	

* – (S) IN MLM = NO GROWTH IN MEDIUM
** – (R) IN MLM = GROWTH IN MEDIUM

TABLE 4–15
EARLY RESULTS OF ANTIBIOTIC DETECTION TRAILS IN MLM(Continued)

9–1719

SPECIMEN	MEDIUM	ORGANISM	ANTIBIOTIC AND MEDIUM CONCENTRATION (MCG/ML)	MLM RESULTS	KIRBY-BAUER STANDARD RESULTS
URINE	E. COLI	E. COLI	KANAMYCIN 10	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	S	S
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	S	S
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	E. COLI	E. COLI	KANAMYCIN	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	S	S
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	S	S
		POSITIVE CONTROL	NONE	GROWTH	
URINE	KLEB/ ENTEROBACTER	K. PNEUMONIAE	KANAMYCIN 10	S	S
			NITROFURANTOIN 5	R	I
			COLISTIN 10	R	I
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	R	I
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	KLEB/ ENTEROBACTER	ENTEROBACTER LIQUEFACIENS	KANAMYCIN 10	S	S
			NITROFURANTOIN 5	R	I
			COLISTIN 10	I	R
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	S	S
		POSITIVE CONTROL	NONE	GROWTH	
URINE	PSEUDOMONAS	P. AERUGINOSA	KANAMYCIN 10	R	R
			NALIDIXIC ACID	R	R
			COLISTIN 10	R	I
			GENTAMICIN 10	S	S
			TETRACYCLINE 1	R	R
		POSITIVE CONTROL	NONE	GROWTH	

TABLE 4–15
EARLY RESULTS OF ANTIBIOTIC DETECTION TRIALS IN MLM (Continued)

9–1720

SPECIMEN	MEDIUM	ORGANISM	ANTIBIOTIC AND MEDIUM CONCENTRATION (MCG/ML)	MLM RESULTS	KIRBY-BAUER STANDARD RESULTS
URINE	STAPHYLOCOCCUS	S. AUREUS NO. 1	PENICILLIN 10	S	S
			TETRACYCLINE 10	S	S
			ERYTHROMYCIN 5	S	S
			CLINDAMYCIN 2	S	S
			OXACILLIN 1	S	S
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	STAPHYLOCOCCUS	S. AUREUS NO. 2	PENICILLIN 10	S	S
			TETRACYCLINE 10	R	R
			ERYTHROMYCIN 5	S	S
			CLINDAMYCIN 2	S	S
			OXACILLIN 1	S	I
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	FECAL STREP	OLD γ STREP	PENICILLIN 10	S	I
			ERYTHROMYCIN 5	S	S
			TETRACYCLINE 10	S	S
			GENTAMICIN 10	R	R
			CLINDAMYCIN	R	R
		POSITIVE CONTROL	NONE	GROWTH	
URINE	FECAL STREP	γ ENTEROCOCCUS	PENICILLIN 10	S	I
			ERYTHROMYCIN 5	S	S
			TETRACYCLINE 10	S	I
			GENTAMICIN 10	R	R
			CLINDAMYCIN 2	R	R
		POSITIVE CONTROL	NONE	GROWTH	
URINE	MIMA-HERELLA	MIMA POLYMORPHA	KANAMYCIN 10	S	S
			NALIDIXIC ACID	R	I
			COLISTIN 10	S	S
			GENTAMICIN 10	S	S
			TETRACYCLINE 1	S	S
		POSITIVE CONTROL	NONE	GROWTH	
URINE	MIMA-HERELLEA	HERELLEA VAGINICOLA	KANAMYCIN 10	S	S
			NALIDIXIC ACID	R	I
			COLISTIN 10	S	S
			GENTAMICIN 10	S	S
			TETRACYCLINE 1	R	R
		POSITIVE CONTROL	NONE	GROWTH	
THROAT	HEMOPHILUS	HEMOPHILUS INFLUENZAE	TETRACYCLINE 10	R	S
			GENTAMICIN 10	R	R
			KANAMYCIN 10	R	R
			COLISTIN 10	S	S
		POSITIVE CONTROL	NONE	GROWTH	

TABLE 4–15
EARLY RESULTS OF ANTIBIOTIC DETECTION TRIALS IN MLM (Continued)

9–1721

SPECIMEN	MEDIUM	ORGANISM	ANTIBIOTIC AND MEDIUM CONCENTRATION (MCG/ML)	MLM RESULTS	KIRBY-BAUER STANDARD RESULTS
URINE	SALMONELLA	S. PARATYPHI B	KANAMYCIN 10	S	S
			NALIDIXIC ACID	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	R	I
			TETRACYCLINE 1	BUBBLES	I
		POSITIVE CONTROL	NONE	GROWTH	
URINE	SALMONELLA	S. TYPHOSA	KANAMYCIN 10	S	S
			NALIDIXIC ACID	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	R	I
			TETRACYCLINE 1	S	S
		POSITIVE CONTROL	NONE	GROWTH	
FECES	SALMONELLA	S. SCHOTTMUELLERI	KANAMYCIN 10	S	S
			NALIDIXIC ACID	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	S	S
			TETRACYCLINE 1	S	I
		POSITIVE CONTROL	NONE	GROWTH	
URINE	COLIFORM	E. COLI	KANAMYCIN 10	S	S
			NITROFURANTOIN 5	S	S
			COLISTIN 10	R	I
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	S	S
		POSITIVE CONTROL	NONE	GROWTH	
URINE	COLIFORM	KLEBSIELLA PNEUMONIAE	KANAMYCIN 10	S	S
			NITROFURANTOIN 5	R	I
			COLISTIN 10	R	I
			GENTAMICIN 10	S	S
			NALIDIXIC ACID	R	I
		POSITIVE CONTROL	NONE	GROWTH	

9–1733

ORGANISM: KLEBSIELLA PNEUMONIAE
SPECIMEN: THROAT
MLM MEDIUM: KLEBSIELLA/ENTEROBACTER BROTH

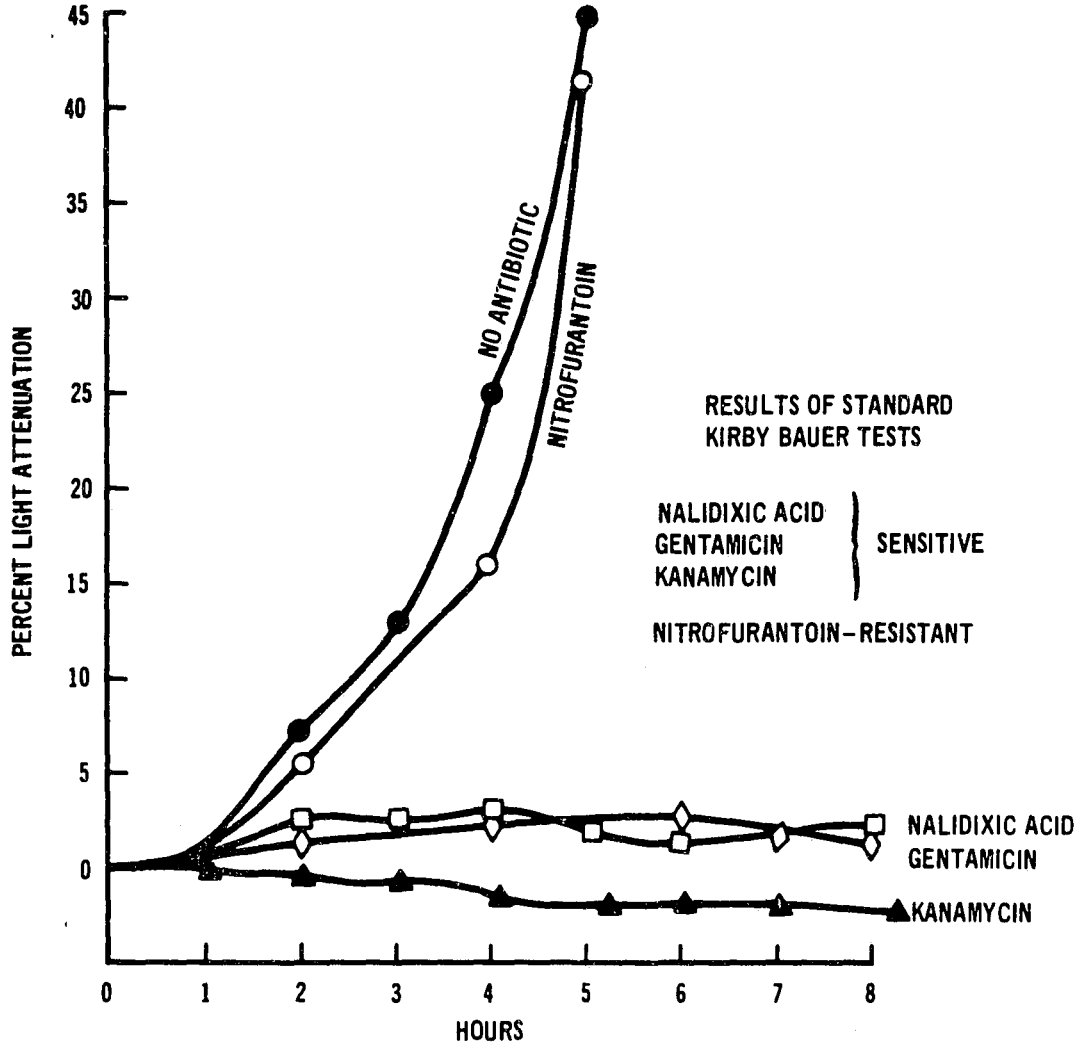


FIGURE 4–1
MLM DETECTION OF ANTIBIOTIC SENSITIVITY

9–1732

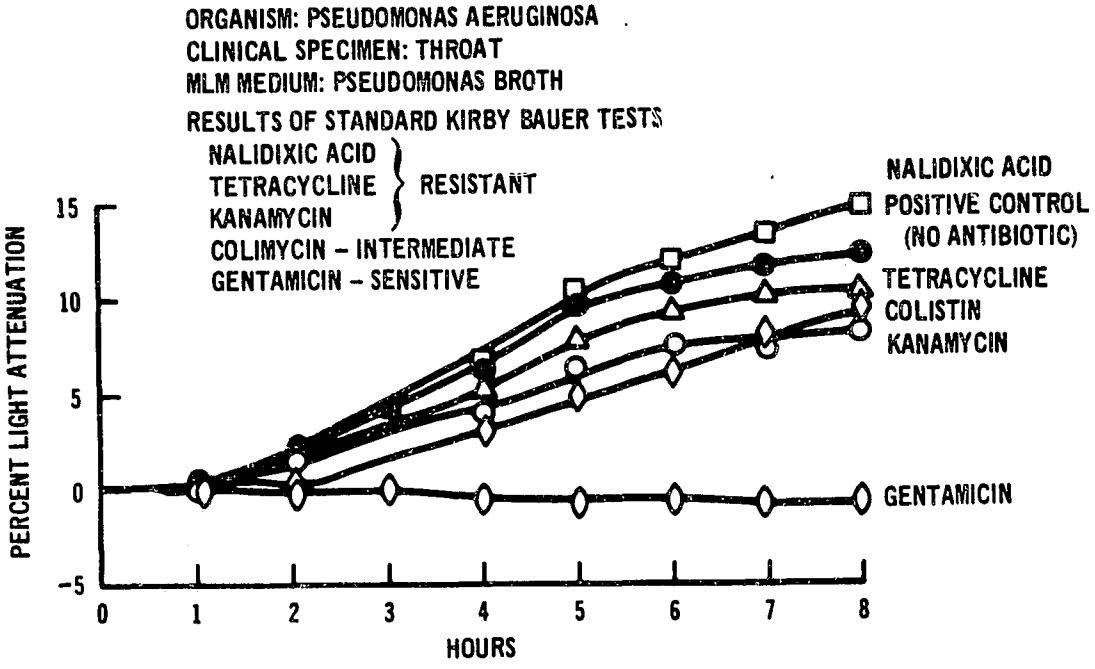


FIGURE 4–2
MLM DETECTION OF ANTIBIOTIC SENSITIVITY

in slow freeze B, Table 4-20, the freezing rate was -.33°C per minute. In each case, the samples were removed at -40°C and transferred to the Revco Ultra Low Temperature Freezer at -80°C for long-term storage testing. The slow freeze storage tests were only carried out for 60 days because comparison with the quick freeze testing showed initial greater damage to the organisms using the slow freeze methods.

Table 4-22 shows microorganism susceptibility to the different freezing processes following one day storage. The quick freeze process produced no immediate loss of organisms while each of the slow freeze processes killed from one to six logs of organisms.

MICROBIAL LOAD MONITOR

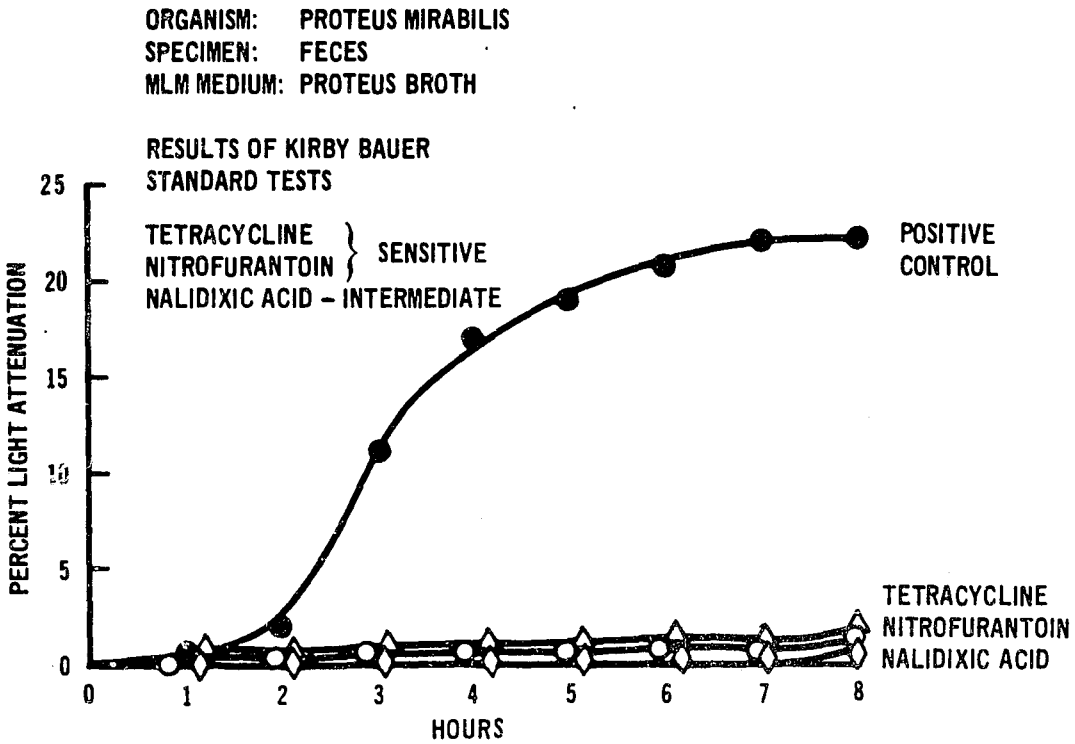


FIGURE 4–3
MLM DETECTION OF ANTIBIOTIC SENSITIVITY

9–1731

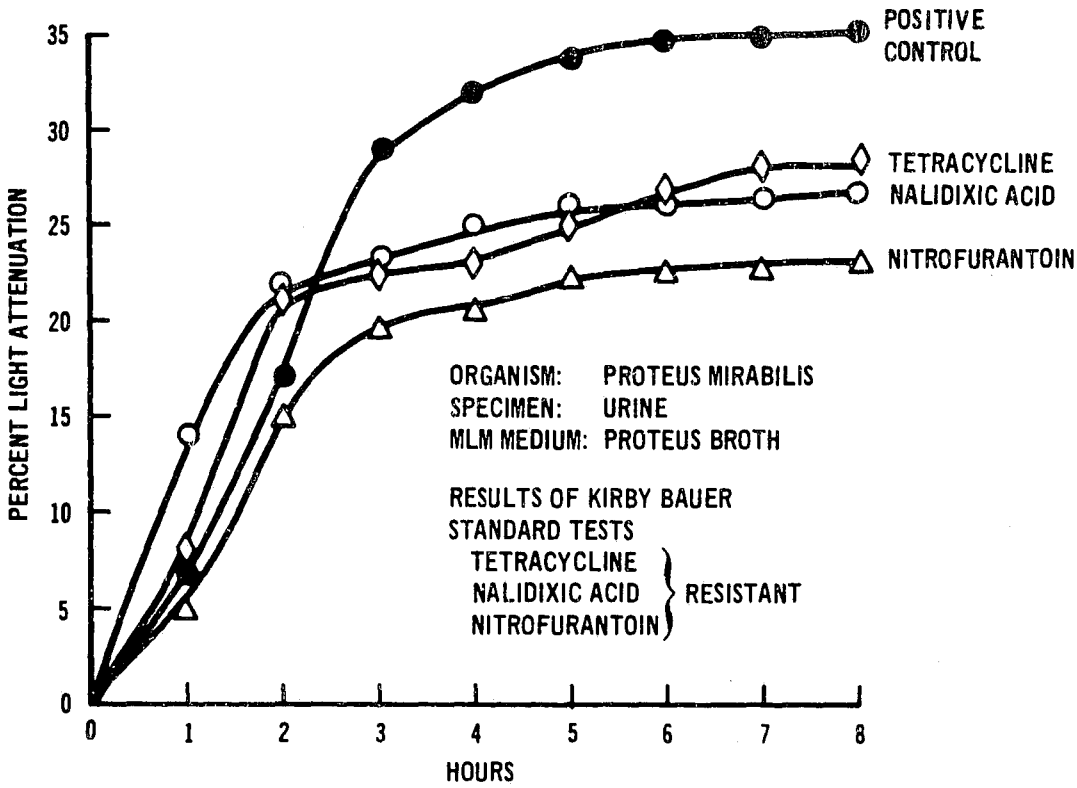


FIGURE 4–4
MLM DETECTION OF ANTIBIOTIC SENSITIVITY

TABLE 4–16
DEVELOPMENTAL SUMMARY OF SELECTIVE MEDIA ANTIMICROBIAL TESTS VS KIRBY BAUER (630 STRAINS)

Antimicrobial – Medium	Total No. of Tests	Agreement	Discrepancies						A Agreement Major & Minor Discrepancies	B Agreement Major Discrepancies Only
			Major							
			MLM KB S R		MLM KB R S		MLM KB R I	Minor MLM KB S I		
Nitrofurantoin – E. coli	200	200	0		0		0	0	100%	100%
Trimethoprim – Sulfa – E. coli	200	199	0		0		0	1	99%	100%
Nalidixic Acid – E. coli	200	200	0		0		0	0	100%	100%
Ampicillin – E. coli (200 Strains)	200	197	1		0		1	1	98.5%	99%
Gentamicin – Klebsiella/Enterobacter	150	150	0		0		0	0	100%	100%
Trimethoprimsulfamethoxazole Klebsiella/Enterobacter	150	144	1		3		0	2	96%	97%
Nalidixic Acid Klebsiella/Enterobacter	150	136	2		3		2	7	90.6%	95.3%
Tetracycline Klebsiella/Enterobacter (150 Strains)	150	133	4		2		0	11	88.6%	96%
Kanamycin – Proteus	150	134	1		13		2	0	89%	89%
Trimethoprimsulfamethoxazole – Proteus	150	134	5		8		0	3	89%	91%
Nalidixic Acid – Proteus	150	122	3		0		0	25	81.3%	98%
Ampicillin – Proteus (150 Strains)	150	138	11		0		0	1	92%	92.6%
Amikacin* – Pseudomonas	100	95	1		1		2	1	95%	96%
Tobramycin* – Pseudomonas	100	98	2		0		0	0	98%	98%
Gentamicin – Pseudomonas	130	129	0		1		0	0	99.2%	99.2%
Carbenicillin – Pseudomonas (130 Strains)	130	122	3		2		3	0	93.8%	93.8%

* 100 Strains

S – Sensitive
R – Resistive
I – Intermediate
Agreement – Complete correlation with Kirby Bauer

% Agreement (Correlation) $A = \frac{\text{No. Agreements}}{\text{Total No. of Tests}} \times 100$

% Agreement (Correlation) $B = \frac{\text{No. Agreement + No. Minor Discrepancies}}{\text{Total No. of Tests}} \times 100$

TABLE 4–17

DEVELOPMENTAL RESULTS – ANTIMICROBIAL SUCEPTIBILITY
RECENTLY OPTIMIZED MEDIA FORMULATIONS

Antimicrobial – Medium	Total No. of Tests	Agreement	Discrepancies				A Agreement Major & Minor Discrepancies	B Agreement Major Discrepancies Only
			Major			Minor		
		S–S R–R	MLM KB S R	MLM KB R S	MLM KB R I	MLM KB S I		
Nitrofurantoin – C. freundi	50	50	0	0	0	0	100%	100%
Nalidixic Acid – C. freundi	50	49	0	1	0	0	98%	98%
Tetracycline – C. freundi	50	50	0	0	0	0	100%	100%
Trimethoprimsulfamethoxazole–C.freundii	50	49	0	1	0	0	98%	98%
Gentamicin – Serratia	50	50	0	0	0	0	100%	100%
Nalidixic Acid – Serratia	50	48	0	2	0	0	96%	96%
Kanamycin – Serratia	50	50	0	0	0	0	100%	100%
Trimethoprimsulfamethoxazole – Serratia	50	50	0	0	0	0	100%	100%
Cephalothin – S.aureus	50	48	1	1	0	0	96%	96%
Tetracycline – S. aureus	50	50	0	0	0	0	100%	100%
Clindamycin – S. aureus	50	50	0	0	0	0	100%	100%
Erythromycin – S. aureus	50	50	0	0	0	0	100%	100%
Kanamycin – Acineto. – Herellea	50	50	0	0	0	0	100%	100%
Nalidixic Acid – Acineto. – Herellea	50	50	0	0	0	0	100%	100%
Gentamicin – Acineto. – Herellea	50	50	0	0	0	0	100%	100%
Tetracycline – Acineto. – Herellea	50	50	0	0	0	0	100%	100%
Nitrofurantoin – Grp D enterococcus	50	50	0	0	0	0	100%	100%
Erythromycin – Grp D enterococcus	50	48	0	0	0	2	96%	100%
Tetracycline – Grp D enterococcus	50	50	0	0	0	0	100%	100%
Ampicillin – Grp D enterococcus	50	50	0	0	0	0	100%	100%

$$\% \text{ Agreement (Correlation) } A = \frac{\text{No. Agreements}}{\text{Total No. of Tests}} \times 100$$

$$\% \text{ Agreement (Correlation) } B = \frac{\text{No. Agreement + No. Minor Discrepancies}}{\text{Total No. of Tests}} \times 100$$

TABLE 4–18
QUICK FREEZE STORAGE AND SURVIVAL TESTS
(No Cryoprotective Agents)

9–1729

ORGANISM	ORGANISMS SURVIVING						
	0 TIME	1 DAY	7 DAYS	30 DAYS	60 DAYS	90 DAYS	130 DAYS
DIPLOCOCCUS PNEUMONIAE	10^3	0	0	0	0	0	0
HERELLEA VAGINICOLA	10^8	10^8	10^3	10^8	10^7	10^6	10^6
SALMONELLA TYPHOSA	10^7	10^7	10^7	10^7	10^5	10^3	10^3
PROTEUS MORGANII	10^6	10^6	10^6	10^5	10^3	0	0
PSEUDOMONAS AERUGINOSA	10^8	10^8	10^6	10^8	10^7	10^4	10^3
CANDIDA ALBICANS	10^7	10^7	10^7	10^7	10^6	10^6	10^6
ESCHERICHIA COLI	10^8	10^8	10^8	10^8	10^7	10^7	10^6
ASPERGILLUS NIGER	10^6	10^6	10^6	10^6	10^5	10^5	10^5
NEISSERIA MENINGITIDIS	10^7	10^6	10^5	10^4	10^3	0	0
STAPHYLOCOCCUS AUREUS	10^8	10^8	10^8	10^8	10^8	10^8	10^7
BETA STREPTOCOCCUS	10^7	10^7	10^7	10^7	10^7	10^7	10^6

TABLE 4–19
STORAGE AND RETURN CAPABILITY TESTING – SLOW FREEZE A

METHOD: ORGANISMS GROWN AND FROZEN AT THE RATE OF –1°C PER MINUTE IN SELECTIVE MEDIA.
STORAGE TEMPERATURE: –79°F

9–1728

GROWTH MEDIUM	ORGANISM TESTED	ORGANISMS RECOVERED PER ML. OF BROTH				
		CONTROL* (0 TIME)	1 DAY	7 DAYS	30 DAYS	60 DAYS
HERELLEA BROTH	HERELLEA VAGINICOLA	10^7	10^7	10^6	10^6	10^6
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^8	NO DATA	10^6	10^6	10^5
COLIFORM BROTH	ESCHERICHIA COLI	10^9	10^8	10^7	10^7	10^5
PSEUDOMONAS BROTH	PSEUDOMONAS AERUGINOSA	10^8	10^5	10^4	10^4	10^4
PROTEUS BROTH	PROTEUS MORGANII	10^8	10^7	10^6	10^6	0
SALMONELLA BROTH	SALMONELLA TYPHOSA	10^8	10^7	10^6	10^5	10^4
STREPTOCOCCUS BROTH NO. 1	BETA STREPTOCOCCUS (GROUP A)	10^6	0	0	0	0
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS (COAGULASE POSITIVE)	10^8	10^8	10^8	10^8	10^8
CANDIDA BROTH	CANDIDA ALBICANS	10^7	10^7	10^6	10^6	10^6

*CONTROL NOT FROZEN TO DETERMINE ORGANISM SUSCEPTIBILITY TO FREEZING PROCESS.

TABLE 4–20
STORAGE AND RETURN CAPABILITY TESTING – SLOW FREEZE B

METHOD: ORGANISMS GROWN AND FROZEN AT THE RATE OF –0.33°C PER MINUTE IN SELECTIVE MEDIA
STORAGE TEMPERATURE: –79°F.

9–1727

GROWTH MEDIUM	ORGANISM TESTED	ORGANISMS RECOVERED PER ML. OF BROTH				
		CONTROL* (0 TIME)	1 DAY	7 DAYS	30 DAYS	60 DAYS
HERELLEA BROTH	HERELLEA VAGINICOLA	10^7	10^7	10^7	10^7	10^6
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^8	10^5	10^5	10^5	10^5
COLIFORM BROTH	ESCHERICHA COLI	10^9	10^7	10^7	10^7	10^6
PSEUDOMONAS BROTH	PSEUDOMONAS	10^8	10^6	10^6	10^6	10^5
PROTEUS BROTH	PROTEUS MORGANII	10^8	10^7	10^7	10^7	0
SALMONELLA BROTH	SALMONELLA TYPHOSA	10^8	10^6	10^5	10^5	10^3
STREPTOCOCCUS BROTH NO. 1	BETA STREPTOCOCCUS (GROUP A)	10^6	0	0	0	0
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS (COAGULASE POSITIVE)	10^8	10^8	10^8	10^8	10^8
CANDIDA BROTH	CANDIDA ALBICANS	10^7	10^7	10^7	10^7	10^7

*CONTROL NOT FROZEN TO DETERMINE ORGANISM SUSCEPTIBILITY TO FREEZING PROCESS.

TABLE 4–21
STORAGE AND RETURN CAPABILITY TESTING – SLOW FREEZE C

METHOD: ORGANISMS GROWN AND FROZEN AT THE RATE OF –0.5°C PER MINUTE IN SELECTIVE MEDIA.
STORAGE TEMPERATURE: –79°F

9–1726

GROWTH MEDIUM	ORGANISM TESTED	ORGANISMS RECOVERED PER ML. OF BROTH				
		CONTROL* (0 TIME)	1 DAY	7 DAYS	30 DAYS	60 DAYS
HERELLEA BROTH	HERELLEA VAGINICOLA	10^7	10^7	10^6	10^6	10^6
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^8	10^6	10^6	10^6	10^6
COLIFORM BROTH	ESCHERICHIA COLI	10^9	10^8	10^7	10^7	10^6
PSEUDOMONAS BROTH	PSEUDOMONAS AERUGINOSA	10^8	10^6	10^5	10^5	10^4
PROTEUS BROTH	PROTEUS MORGANII	10^8	10^7	10^7	10^6	0
SALMONELLA BROTH	SALMONELLA TYPHOSA	10^8	10^6	10^6	10^6	10^3
STREPTOCOCCUS BROTH NO. 1	BETA STREPTOCOCCUS	10^6	0	0	0	0
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS (COAGULASE POSITIVE)	10^8	10^8	10^8	10^8	10^8
CANDIDA BROTH	CANDIDA ALBICANS	10^7	10^6	10^6	10^6	10^6

*CONTROL NOT FROZEN TO DETERMINE ORGANISM SUSCEPTIBILITY TO FREEZING PROCESS

TABLE 4–22
STORAGE AND RETURN CAPABILITY TESTING COMPARISON OF QUICK-FREEZE AND SLOW-FREEZE METHOD (1 DAY)

9–1725

GROWTH MEDIUM	ORGANISM TESTED	LOG REDUCTION IN NUMBER OF ORGANISMS AFTER 1 DAY STORAGE AT –79°F			
		QUICK-FREEZE	SLOW-FREEZE A	SLOW-FREEZE B	SLOW-FREEZE C
HERELLEA BROTH	HERELLEA VAGINICOLA	NO LOSS	NO LOSS	NO LOSS	NO LOSS
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	NO LOSS	–	2 LOG	3 LOG
DIPLOCOCCUS BROTH	DIPLOCOCCUS PNEUMONIAE	NO LOSS	–	–	–
COLIFORM BROTH	ESCHERICHIA COLI	NO LOSS	1 LOG	1 LOG	2 LOG
PSEUDOMONAS BROTH	PSEUDOMONAS AERUGINOSA	NO LOSS	3 LOG	2 LOG	2 LOG
PROTEUS BROTH	PROTEUS MORGANII	NO LOSS	1 LOG	1 LOG	1 LOG
SALMONELLA BROTH	SALMONELLA TYPHOSA	NO LOSS	1 LOG	2 LOG	2 LOG
STREPTOCOCCUS BROTH NO. 1	BETA STREPTOCOCCUS (GROUP A)	NO LOSS	6 LOG	6 LOG	6 LOG
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS (COAGULASE POSITIVE)	NO LOSS	NO LOSS	NO LOSS	NO LOSS
CANDIDA BROTH	CANDIDA ALBICANS	NO LOSS	1 LOG	1 LOG	NO LOSS
SYNTHETIC BROTH	ASPERGILLUS NIGER	NO LOSS	–	–	–

Overall loss of organisms following 60 days of storage at -79°F is shown in Table 4-23 for each method. Both quick freeze and slow freeze processes yielded similar overall loss of organisms after 60 days storage. Even though the different processes yielded similar overall results the quick freeze process is advantageous because it is the simpler method.

Cryoprotective Agents - Results of storage tests utilizing the three cryoprotective agents previously discussed are shown in Table 4-24. Results of the cryoprotective agent study indicate that any of the three agents would be useful in the preservation of delicate organisms such as Neisseia meningitidis and streptococcus pneumoniae.

TABLE 4–23

STORAGE AND RETURN CAPABILITY TESTING COMPARISON OF QUICK-FREEZE AND SLOW-FREEZE METHOD (60 DAYS)

9–1724

GROWTH MEDIUM	ORGANISM TESTED	LOG REDUCTION IN NUMBER OF ORGANISMS AFTER 60 DAYS STORAGE AT –79°F			
		QUICK FREEZE	SLOW FREEZE A	SLOW FREEZE B	SLOW FREEZE C
HERELLEA BROTH	HERELLEA VAGINICOLA	1 LOG	1 LOG	1 LOG	1 LOG
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	3 LOG	3 LOG	2 LOG	3 LOG
DIPLOCOCCUS BROTH	DIPLOCOCCUS PNEUMONIAE	1 LOG	–	–	–
COLIFORM BROTH	ESCHERICHIA COLI	NO LOSS	4 LOG	3 LOG	3 LOG
PSEUDOMONAS BROTH	PSEUDOMONAS AERUGINOSA	NO LOSS	4 LOG	4 LOG	3 LOG
PROTEUS BROTH	PROTEUS MORGANII	6 LOG	8 LOG	8 LOG	8 LOG
SALMONELLA BROTH	SALMONELLA TYPHOSA	6 LOG	4 LOG	5 LOG	5 LOG
STREPTOCOCCUS BROTH NO. 1	BETA STREPTOCOCCUS (GROUP A)	6 LOG	6 LOG	6 LOG	6 LOG
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS (COAGULASE POSITIVE)	NO LOSS	NO LOSS	NO LOSS	NO LOSS
CANDIDA BROTH	CANDIDA ALBICANS	NO LOSS	1 LOG	1 LOG	1 LOG
SYNTHETIC BROTH	ASPERGILLUS NIGER	6 LOG	–	–	–

Freeze Drying Techniques - Results from tests using freeze drying as a means of preserving microorganisms showed that the lyophilization process itself was quite detrimental to the recovery of less hardy organisms such as Neisseria meningitidis and streptococcus pneumoniae. Results are shown in Table 4-25. The addition of cryoprotective agents before lyophilization had no significant effect on these organisms, and in the case of S. aureus, decreased the survival rate even more than when no cryoprotective agents were used.

Recommendations for Storage and Return of Microorganisms

a. Freeze all microorganisms with the exceptions of N. meningitidis and S. pneumoniae in their selective media. Freezing should occur as rapidly as possible.

b. N. meningitidis and S. pneumoniae should be mixed with a cryoprotective agent before freezing.

c. Storage temperatures should be maintained below -40°C. Survival rates increase slightly as the temperature is lowered to -80°C.

TABLE 4–24
CRYOPROTECTIVE AGENT STUDIES

RESULTS OF STORAGE TESTS AT –79° F

9–1723

		APPROXIMATE SURVIVING ORGANISMS (IN LOGS)				
		0 TIME	1 DAY	1 WEEK	60 DAYS	130 DAYS
I. POLYVINYLPYRROLIDINONE MW 40,000 15% W/V						
MEDIUM	ORGANISM					
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS	10^8	10^8	10^8	10^8	10^8
DIPLOCOCCUS BROTH	DIPLOCOCCUS PNEUMONIAE	10^5	10^5	10^5	10^5	10^5
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^5	10^4	10^4	10^3	10^2
II. GLYCEROL 15% W/V						
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS	10^8	10^8	10^8	10^8	10^7
DIPLOCOCCUS BROTH	DIPLOCOCCUS PNEUMONIAE	10^6	10^6	10^6	10^6	10^5
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^6	10^4	10^4	10^2	10^2
III. DIMETHYL SULFOXIDE 10% W/V						
STAPHYLOCOCCUS BROTH	STAPHYLOCOCCUS AUREUS	10^8	10^8	10^8	10^8	10^7
DIPLOCOCCUS BROTH	DIPLOCOCCUS PNEUMONIAE	10^6	10^6	10^6	10^6	10^5
NEISSERIA BROTH	NEISSERIA MENINGITIDIS	10^6	10^4	10^4	10^3	10^2

4.1.6 Clinical and Seeded Samples - Results - Early clinical and seeded samples results have been well documented (MDC E0727, December 1972; MDC E0317, March 1971; MDC E098, 7 January 1974) and will not be reproduced here. These tests represent several thousand media-organism combinations and challenges. As seen from Table 4-26, a summary of these early results indicated 94% to 100% agreement with standard test results; average detection times ranged from 4.6 to 15.7 hours for the three types of clinical samples evaluated.

TABLE 4–25
FREEZE DRYING STUDIES

9–1722

AGENT	SURVIVING ORGANISMS IN LOGS						
	0 TIME	1 DAY	7 DAYS	30 DAYS	60 DAYS	90 DAYS	130 DAYS
NO AGENT ADDED (CONTROL)							
STAPHYLOCOCCUS AUREUS	10^8	10^7	10^7	10^7	10^7	10^7	10^7
DIPLOCOCCUS PNEUMONIAE	10^6	10^5	0	0	0	0	0
NEISSERIA MENINGITIDIS	10^3	0	0	0	0	0	0
POLYVINYL PYRROLIDINONE 40,000 MW, 15% W/V							
STAPHYLOCOCCUS AUREUS	10^7	0	0	0	0	0	0
DIPLOCOCCUS PNEUMONIAE	0	0	0	0	0	0	0
NEISSERIA MENINGITIDIS	0	0	0	0	0	0	0
GLYCEROL 15% W/V							
STAPHYLOCOCCUS AUREUS	10^8	10^6	10^5	10^3	0	0	0
DIPLOCOCCUS PNEUMONIAE	10^7	$<10^4$	0	0	0	0	0
NEISSERIA MENINGITIDIS	10^4	0	0	0	0	0	0
10% DIMETHYL SULFOXIDE w/v							
STAPHYLOCOCCUS AUREUS	10^8	10^5	10^5	0	0	0	0
DIPLOCOCCUS PNEUMONIAE	10^6	$<10^4$	0	0	0	0	0
NEISSERIA MENINGITIDIS	10^4	$<10^3$	0	0	0	0	0

NOTE:
ORGANISMS GROWN IN SELECTIVE MEDIA THEN ADDED TO CRYOPROTECTIVE AGENTS AND FREEZE DRIED. STORAGE WAS -79^0 F

Many of the media first tested in the MLM were applied to the current McDonnell Douglas commercial version - the AutoMicrobic System. These media formulations are identical for both systems. Although differences exist between the two instruments, both perform the same task, i.e., optical monitoring of these media and automated reporting of results. Developmental data totaling over 8000 clinical and seeded urine samples have been reported (10,11). Agreement with simultaneous conventional (manual) cultures at levels of 70,000 colony-forming units per ml (or more) was 92% or better for seeded specimens; clinical specimens yielded results of 93% or better for all organisms except P. aeruginosa, where agreement was 86%. The media evaluated in these tests are listed in Table 4-27. Several MLM media are not utilized in the AutoMicrobic System. These include Acinetobacter-Herellea, Beta streptococcus, and fungi broths.

9–1660

TABLE 4–26

DETECTION OF ORGANISMS IN MLM CLINICAL TRIALS EARLY STUDIES – SUMMARY OF RESULTS

(150 Challenges per Medium)

Medium – Sample	% Agreeing with Standard Tests	Average Detection Time in Hours
Proteus – Throat	96%	5.2
Proteus – Urine	94%	7.2
Proteus – Feces	98%	4.6
P. aeruginosa – Throat	96%	5.1
P. aeruginosa – Urine	94%	4.7
P. aeruginosa – Feces	94%	7.6
Klebsiella – Enterobacter – Throat	96%	5.4
Klebsiella – Enterobacter – Urine	98%	8.3
Klebsiella – Enterobacter – Feces	98%	7.8
Coliform – Throat	98%	5.6
Coliform – Urine	100%	4.8
Coliform – Feces	96%	7.4
E. coli – Throat	96%	11.5
E. coli – Urine	100%	11.4
E. coli – Feces	94%	12.1
Salmonella – Throat	92%	4.1
Salmonella – Urine	96%	5.4
Salmonella – Feces	92%	8.5
S. aureus – Throat	100%	7.7
S. aureus – Urine	98%	7.9
S. aureus – Feces	100%	–
Acinetobacter – Herellea – Throat	98%	9.9
Acinetobacter – Herellea – Urine	98%	10.7
Acinetobacter – Herellea – Feces	98%	8.3
Aspergillus (Fungi) – Throat	92%	15.7
Aspergillus (Fungi) – Urine	92%	15.6
Aspergillus (Fungi) – Feces	96%	15.5
C. albicans (Yeast) – Throat	98%	9.4
C. albicans (Yeast) – Urine	100%	8.8
C. albicans (Yeast) – Feces	98%	12.1
S. pneumoniae – Throat	99%	5.8
Group D enterococcus – Throat	98%	3.8
Group D enterococcus – Urine	94%	6.6
Group D enterococcus – Feces	100%	7.8

9-1655

TABLE 4-27
MLM SELECTIVE MEDIA EVALUATED FOR THE AUTOMICROBIC SYSTEM

P. aeruginosa
Proteus SP.
C. freundi
Serratia SP.
E. coli
Klebsiella – Enterobacter
Yeast
Grp D enterococcus
S aureus
Positive Control
Enumeration

Recent MLM Clinical and Seeded Studies - Recent MLM Clinical and Seeded tests are defined as those tests performed using the current deliverable instrumentation and software. As this deliverable MLM has been under construction and test through the majority of the current contract extension period, total number of samples tested represent a small segment of total data reported in this section. Additionally, a maximum of five samples may be tested per day on the MLM whereas previous carousel MLM version allowed 150 sample load and the AutoMicrobic System can process 240 samples on a daily basis.

Overall MLM performance results for the most part are parallel to early media test results and are in good agreement with AutoMicrobic System reports. Results of the recent MLM Clinical and Seeded tests are given in Tables 4-28 and 4-29. Media performance in MLM Cards is extensively covered in Section 4.3, Reliability Tests.

The results in Table 4-28 differ from previous results, and AMS (McDonnell Douglas commercial venture) results for a variety of reasons. Early MLM results (Table 4-26) were taken with cassettes and liquid media, the formulations of which are probably not the same as used now in the MLM. The cassettes have multiple wells from which to determine positives and negatives. Recent MLM data is taken from only one lot of Cards (i.e., one media batch) while AMS data in general is over many production lots after quality control checks. Determination of good media before freeze drying is impossible at this time.

TABLE 4–28
SEEDED & CLINICAL TEST RESULTS – MLM CLINICAL CARD

9–1746

Medium	No. of Challenges	True Positives	True Negatives	False Positives	False Negatives	% Correlation
E. coli	171	41	123	5*	2	96
Klebsiella-Enterobacter	171	23	143	1	4	97
C. freundii	171	7	160	2	2	98
Proteus sp.	171	28	107	0	5**	79
P. aeruginosa	171	9	155	2	5	96
Serratia sp.	171	5	164	1	2	99
S. aureus	171	10	152	7	2	95
Group D Enterococcus	171	39	131	0	1	99
Acinetobacter-Herellea	109	0	102	2	5	94
Grp A Beta Strep	109	0	101	5	3	93
Yeast	171	11	158	0	2	99
Pos. Control ($> 10^2$/ml)	171	147	15	0	9	95
Enumeration	171	124	33	2 (MLM $> 10^5$/ml STD $< 10^5$/ml)	12*** (MLM $< 10^5$/ml STD $\geq 10^5$/ml)	92

$$\% \text{ Correlation} = \frac{\text{True Pos.} + \text{True Neg.}}{\text{Total}} \times 100$$

* C. freundii
** 3 Proteus $\geq 10^8$/ml
*** 4 Bad Fills

TABLE 4–29
SEEDED & CLINICAL TEST RESULTS – MLM CLINICAL ANTIBIOTICS

9–1747

Antimicrobial	Media Used	Total No.of Tests	Agreement MLM=S KB=S	Agreement MLM=R KB=R	Major Discrepancies MLM=S KB=R	Major Discrepancies MLM=R KB=S	Minor Discrepancies MLM=S KB=I	Minor Discrepancies MLM=R KB=I	% Correlation
Nitrofurantoin	E. coli	41	41		0		0		100
	C. freundii	7	7		0		0		100
	Group D. Entero.	39	39		0		0		100
Trimeth-Sulfa	E. coli	41	41		0		0		100
	Kleb-Ent.	23	23		0		0		100
	C. freundii	6	6		0		0		100
	Proteus	25	23		1		1		96
	Serratia	3	3		0		0		100
Nalidixic Acid	E. coli	41	41		0		0		100
	Kleb-Ent.	23	23		0		0		100
	C. freundii	6	6		0		0		100
	Proteus	26	25		1		0		96
	Serratia	3	3		0		0		100
	Acineto-Herellea	0							
Ampicillin	E. coli	41	41		0		0		100
	Proteus	25	22		3		0		88
	Group D. Entero.	39	38		1		0		97
	Group A Beta	0							

KB = Kirby-Bauer (Standard Method)
S = Sensitive
R = Resistive
I = Intermediate
Agreement – Complete Correlation with Kirby Bauer

$$\% \text{ Correlation} = \frac{\text{Agreement}}{\text{Total No. of Tests}} \times 100$$

TABLE 4–29
SEEDED & CLINICAL TEST RESULTS – MLM CLINICAL ANTIBIOTICS (Continued)

9–1750

Antimicrobial	Media Used	Total No.of Tests	Agreement MLM=S KB=S	Agreement MLM=R KB=R	Major Discrepancies MLM=S KB=R	Major Discrepancies MLM=R KB=S	Minor Discrepancies MLM=S KB=i	Minor Discrepancies MLM=R KB=I	% Correlation
Gentamicin	Kleb-Ent.	23	22		1		0		96
	Pseudomonas	9	9		0		0		100
	Serratia	3	3		0		0		100
	Acineto-Herellea	0							
Tetracycline	Kleb-Ent.	23	20		2		1		91
	C. freundii	7	7		0		0		100
	S. aureus	10	10		0		0		100
	Group D. Entero.	39	37		1		1		97
	Acineto-Herellea	0							
Kanamycin	Proteus	25	22		3		0		88
	Serratia	3	3		0		0		100
	Acineto-Herellea	0							
Amikacin	Pseudomonas	9	9		0		0		100
Tobramycin	Pseudomonas	9	9		0		0		100
Carbenicillin	Pseudomonas	9	9		0		0		100
Erythromycin	Group D. Entero	39	33		1		5 (KB=I MLM=S)		97
	Group A Beta	0							
Clindamycin	S. aureus	10	10		0		0		100

4.1.7 Quality Control and Shelf-Life Studies

Sterility Tests - Assembled SRCLDs - Results of sterility tests are given in Table 4-30. Based on test results, all remaining assembled SRCLDs were subjected to electron beam sterilization. Although the < 50 cfu/ml count obtained on unsterilized controls may not alter selective media performance, these numbers could give positive results with the general enrichment enumeration media, and thereby alter enumeration results.

TABLE 4–30
RESULTS – STERILITY TESTS OF ASSEMBLED SRCLD'S

9–1654

	Results			
	Test 1	Test 2	Test 3	Test 4
Assembled – No electronbeam Sterilization	32 CFU/mL	N. G.	15 CFU/mL	40 CFU/mL
Assembled – 2 mr Sterilization	N. G.	N. G.	N. G.	N. G
Assembled – 3 mr Sterilization	N. G.	N. G.	N. G.	N. G.

N. G. = No growth observed 72 hrs.
35°C

Sterility Tests - Completed Cards - Sterility tests on completed and packaged Cards were conducted at the same time as quality control (performance) tests. A minimum of five Cards were filled with sterile 0.5% NaCl and allowed to incubate for evidence of growth. With few exceptions, most lots of prepared Cards were shown to have no signs of contamination. In those cases of contamination, the most commonly found organism was Bacillus sp. and the medium most often involved was the general-purpose positive control, enumeration formulation.

Quality Control (Performance) Tests - MLM Cards - Performance tests of completed MLM Cards were performed as described in Section 3. Results are given in Table 4-31 for those MLM Clinical Cards produced for NASA delivery under terms of this contract. Quality control test results for delivered MLM Environmental Cards are given in Table 4-32. MLM Clinical Cards delivered at the close of this contract have been continually tested during a 5-1/2 month time period. Clinical and seeded results obtained during this period indicate no decrease in performance of these Cards for refrigerated storage.

TABLE 4–31
QUALITY CONTROL TESTS – SUMMARY
MLM CLINICAL CARDS

9–1791

Organism		Count CFU/ml	Positive Media	Susceptibility Test Discrepancies	Positive Control	Enumeration (# Pos. Wells)
P. aeruginosa	30	6.2×10^5	Pseudomonas	None	Yes	5
P. aeruginosa	43	5.7×10^5	Pseudomonas	None	Yes	No Fill
P. aeruginosa	70	1.0×10^6	Pseudomonas Acineto-Herellea*	None	Yes	5
P. aeruginosa	53	5.2×10^5	Pseudomonas	None	Yes	5
P. aeruginosa	59	4.3×10^5	None	None	No*	5
P. aeruginosa	61	4.1×10^5	Pseudomonas	None	Yes	5
E. coli	1	6.2×10^5	E. coli	None	Yes	5
E. coli	2	4.8×10^6	E. coli	None	Yes	5
C. freundii	1	6.1×10^5	E. coli; C. freundii	None	Yes	5
C. freundii	2	4.2×10^5	C. freundii	None	Yes	5
C. freundii	3	6.0×10^5	E. coli	None	Yes	5
C. albicans	1	8.0×10^5	Yeast	N.A.	Yes	0*
C. albicans	2	4.0×10^5	Yeast	N.A.	Yes	4
S. marcescens	1	1.0×10^6	Serratia	None	Yes	5
S. marcescens	2	8.6×10^5	Serratia	None	Yes	5
S. liquefaciens	1	7.1×10^5	Serratia	None	Yes	5
S. liquefaciens	2	2.8×10^5	None*	–	Yes	5
P. mirabilis	4	1.2×10^6	Proteus	None	Yes	5
P. morganii	1	1.0×10^6	Proteus	None	Yes	5
P. morganii	2	5.1×10^5	Proteus	None	Yes	5
K. pneumoniae	1	4.2×10^4	Late K/E	None	Yes	3
K. pneumoniae	2	6.4×10^5	K/E	None	Yes	5
E. aerogenes	1	5.6×10^5	K/E	None	Yes	5
S. aureus	1	7.1×10^5	S. aureus	None	Yes	5
S. aureus	2	6.5×10^5	S. aureus	None	Yes	5
S. aureus	3	1.4×10^6	S. aureus	None	Yes	5
S. aureus	4	8.1×10^4	None*	–	Late	0
Grp D Entero	26	6.1×10^5	Grp D Entero	None	Yes	5
Grp D Entero	20	7.2×10^5	Grp D Entero	None	Yes	5
Grp D Entero	29	5.2×10^5	Grp D Entero	Te – Late MLM=R KB=S	Yes	5
Acinetobacter sp	1	5.8×10^4	None*	–	No	0
Acinetobacter sp	2	1.6×10^5	None*	–	No	0
Herellea vaginicola	1	5.1×10^5	15 Hr A-H	–	Yes	0
Grp A Beta strep	1	1.4×10^5	None*	–	Yes	0
Grp A Beta strep	4	4.1×10^5	None*	–	No	0
Sterile fill		–	None	–	No Growth	No Growth
Sterile fill		–	None	–	No Growth	No Growth
Sterile fill		–	None	–	No Growth	No Growth

*Failure

TABLE 4-32
QUALITY CONTROL TESTS SUMMARY
MLM ENVIRONMENTAL CARDS

9-1748

Organism		Count (CFU/ml)	Positive Media	Positive Control
E. coli	1	2×10^3	E. coli 15 hr	Yes
E. coli	2	1.5×10^6	E. coli, Beta strep	Yes
K. pneumoniae	1	7.6×10^5	K-E, Beta	Yes
K. pneumoniae	2	4.1×10^5	K-E, Yeast*	Yes
E. aerogenes	1	1.4×10^6	K-E, Beta strep	Yes
P. mirabilis	1	2.0×10^5	Proteus	Yes
P. mirabilis	2	1.4×10^6	Proteus, Beta strep	Yes
P. morganii	1	7.2×10^5	Proteus	Yes
S. marcescens	1	1.8×10^6	Serratia	Yes
S. liquefaciens	1	4.6×10^5	None	Yes
C. freundii	1	8×10^5	C. freundii	Yes
C. freundii	2	6.8×10^4	C. freundii 15 hr	Yes
Grp D Enterococcus	1	5.1×10^5	Group D Enterococcus	Yes
Grp D Enterococcus	2	6.2×10^5	Group D Enterococcus	Yes
S. aureus	1	5.8×10^5	S. aureus 15 hr	Yes
S. aureus	2	7.4×10^5	S. aureus	Yes
Acinetobacter sp	1	1.6×10^6	Acineto-Her. – Pseudomonas	Yes
Herellea vaginicola	1	5.6×10^5	Acineto-Her. – Pseudomonas	Yes
P. aeruginosa	1	5.8×10^5	Pseudomonas	Yes
Grp A Beta strep	1	1.6×10^6	Beta strep	Yes
Grp A Beta strep	2	4.2×10^6	Beta strep 15 hr	Yes
Aspergillus niger		Undetermined	Fungi 18 hr	Yes
Sterile fill		–	None	Negative
Sterile fill		–	None	Negative
Sterile fill		–	None	Positive

*Failure

Shelf-Life Studies - Long-term shelf-life studies have been conducted on the AMS-MLM formulations listed in Table 4-32. Performance results on Cards maintained for one year at refrigerated storage are shown in Table 4-33. As shown, no significant differences were noted on stored media (12 months) versus new media. Accelerated shelf-life studies have also been performed. Results indicate good stability of the selective media held at room temperature for extended time periods of several months. However, those media containing antimicrobials have shown decreased shelf life under ambient conditions. It is recommended, therefore, that MLM Clinical Cards be stored under refrigeration.

Formal shelf-life studies for all antimicrobial-media combinations used in the MLM Clinical Card are not complete beyond six months. Data show that stability is maintained for a minimum of six months of refrigerated storage. One year refrigerated shelf-life studies for several AMS media have been completed at this time and test results indicate good performance for Cards refrigerated for this period of time.

TABLE 4–33
PERFORMANCE RESULTS – SELECTIVE MEDIA
0 TIME VS 12 MO. STORAGE 4°C

9–1749

Organism*	No. Strains Tested	Positive Media		Average Detection Time		False Positive (AMS Call)		False Negative	
		0 Time	12 Mo.	0 Time	12 Mo.	0 Time	12 Mo.	0 Time	12 Mo.
P. aeruginosa	5	Pseudomonas	Pseudomonas	9.5 h	9.5 h	None	None	None	None
Proteus sp	5	Proteus	Proteus	8 h	7 h	One Strain Pseudomonas	None	None	None
C. freundii	3	C. freundii E. coli	C. freundii E. coli	9 h	8 h	E. coli	None	None	None
S. marcescens	5	Serratia	Serratia	10 h	10 h	One Strain Proteus	None	None	None
E. coli	5	E. coli	E. coli	7 h	7 h	None	None	None	None
K. pneumoniae	2	K/E	K/E	8.5 h	9 h	None	S. aureus (Bubble)	None	None
Enterobacter sp	4	K/E	K/E	9 h	9 h	None	None	None	None
Candida sp	5	Yeast	Yeast	8.5 h	9 h	None	None	None	None
S.aureus	5	S. aureus	S. aureus	10.5 h	10.5 h	None	None	None	None
Grp D Entero	5	Grp D Entero	Grp D Entero	9 h	8 h	One E. coli	None	None	None
Pos Control	44	Pos Control	Pos Control	6 h	6 h	None	None	None	None
Enumeration	44	Enumer.	Enumer.	–	–	–	–	2 Yeasts	None

*All organisms seeded at 10^5 CFU/ml

4.2 HARDWARE AND SOFTWARE RESULTS

Major results presented in this section are as follows: (1) Cassette/Card; (2) Sample Loading Equipment; (3) Incubation and Detection Instrument; (4) Ancillary Equipment; and (5) Software.

4.2.1 Cassette/Card - The heart of the Microbial Load Monitor System is the cassette or Card in which the selective media are loaded, inoculated, incubated, and monitored for optical changes. The development process has seen many versions of the cassette/Card which proved useful for a portion of the study. The two versions worthy of note are the cassette(both antibiotic and dual) and the Card.

4.2.1.1 Cassette - The plastic cassette was designed for simplicity of use and production. Three versions were designed and used with the latter two produced in large quantity. These are the filter cassette, Figure 4-5; the combination cassette, Figure 4-6; and the dual cassette, Figure 4-7. Molded cassettes were produced by the thousands and were used extensively for the biological and engineering tests. The cassettes were flexible enough to be used with or without filters. Without filters, the basic cassette design became the test bed for a multiple detection cassette and a forerunner of the integrated cassette. Production of the cassette was simple and reliable until freeze drying of media in the cassette was attempted. Previously only taping, septums and maybe filters were required. Media was introduced in the liquid form. With freeze drying of media during Card production, emphasis on exact metering of media into each well, proper freeze drying, taping after freeze drying and pouching to prevent rehydration would be required. Final taping and pouching would have to be performed in a dry atmosphere. These added requirements increased the complexity of the processing procedures.

Two significant problems developed with the cassette design. The first problem was that the cassette with its loading device was prone to leaving or generating bubbles within the viewing area. Also, the cassette loading and handling was not simple when an inoculum was to be tested against many medias. Since the inoculum of the combination or dual cassette flowed through all media wells, only one media type could be used. Majority voting on four identical wells was used with this cassette. The integrated cassette/Card and loading system alleviated the bubble problem and the integrated cassette/Card does not have any flow through wells, so different media in each well can be used.

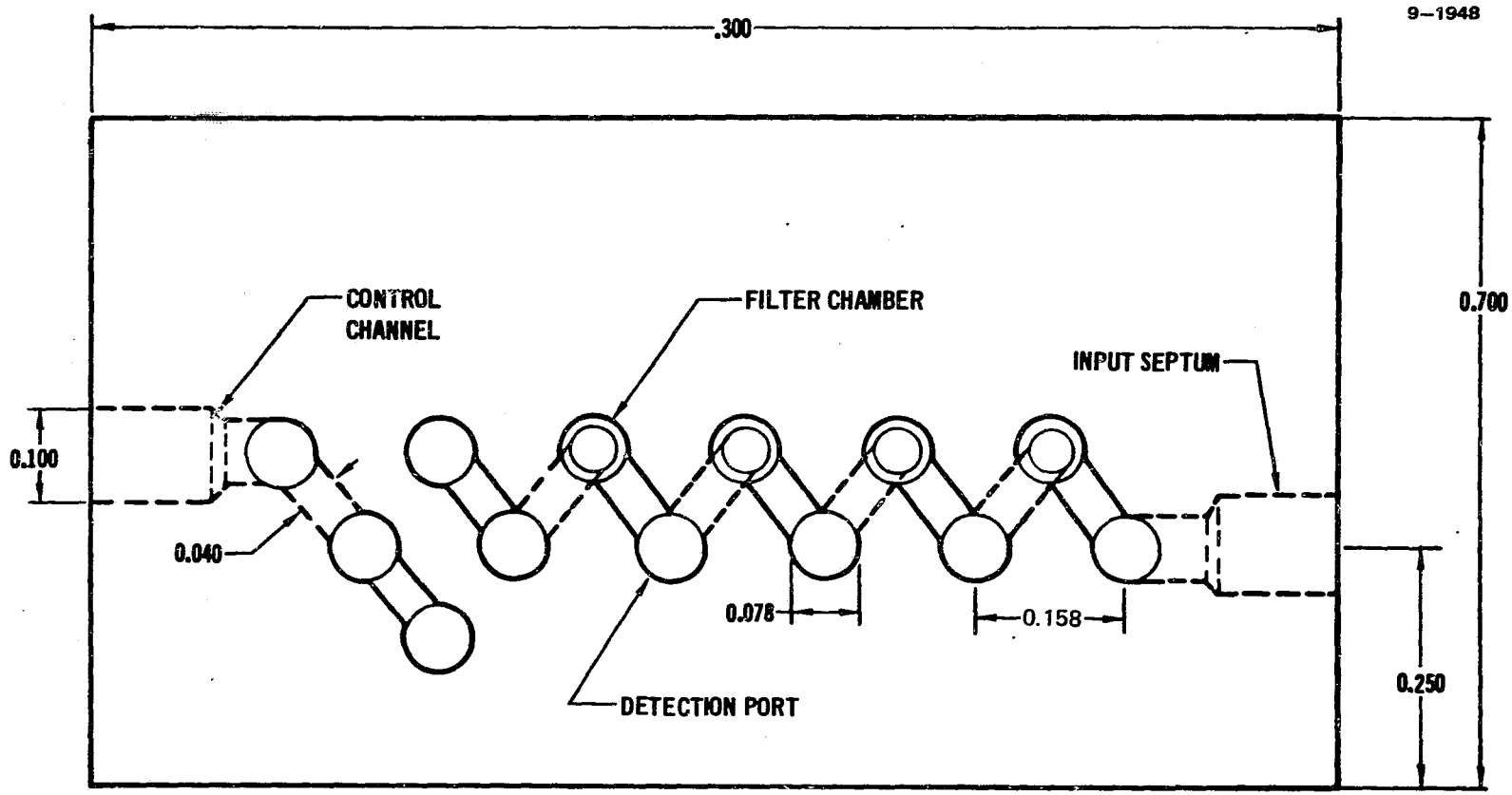


FIGURE 4–5
FIVE CHANNEL FILTER CASSETTE

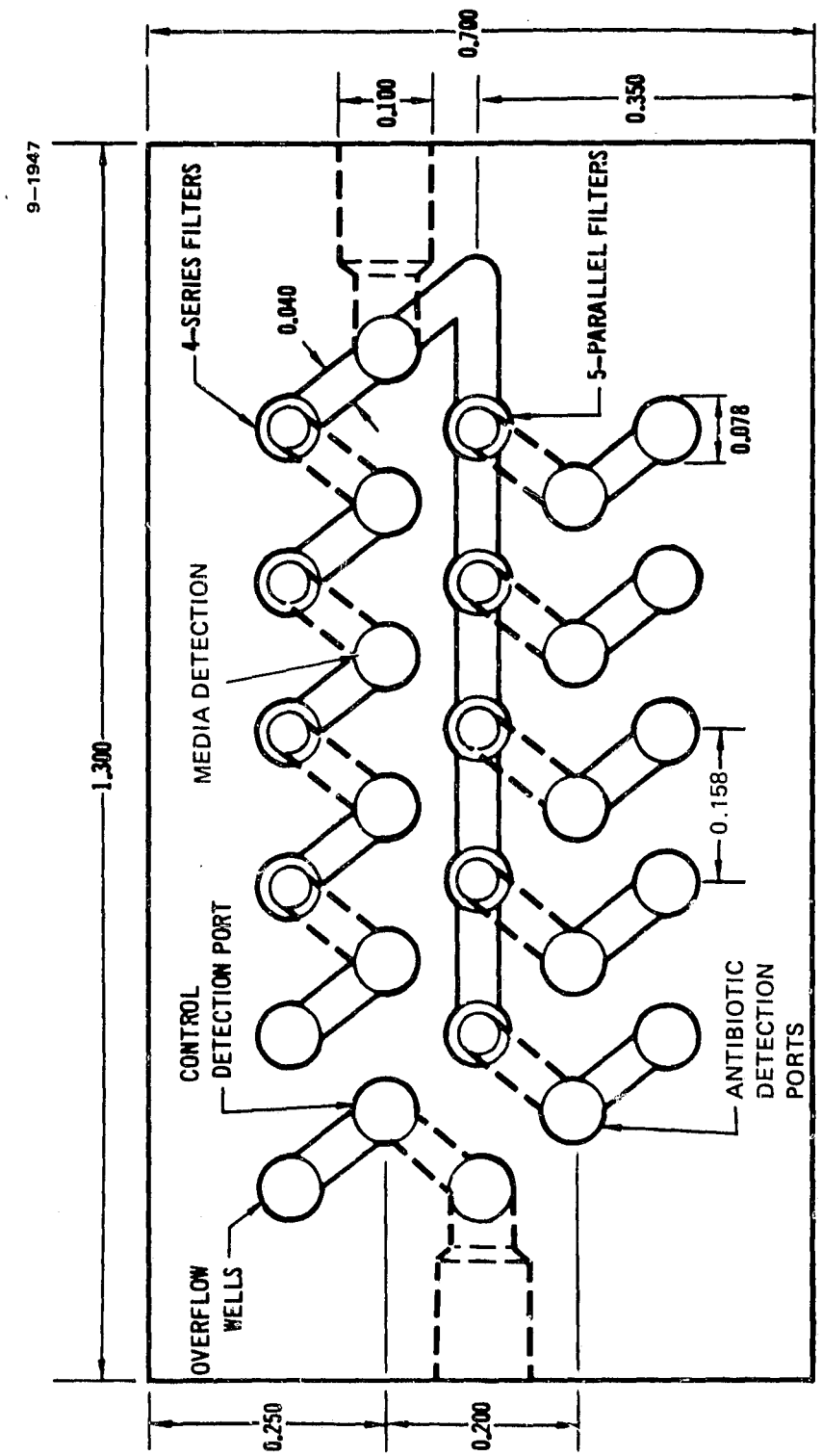


FIGURE 4-6
COMBINATION CASSETTE

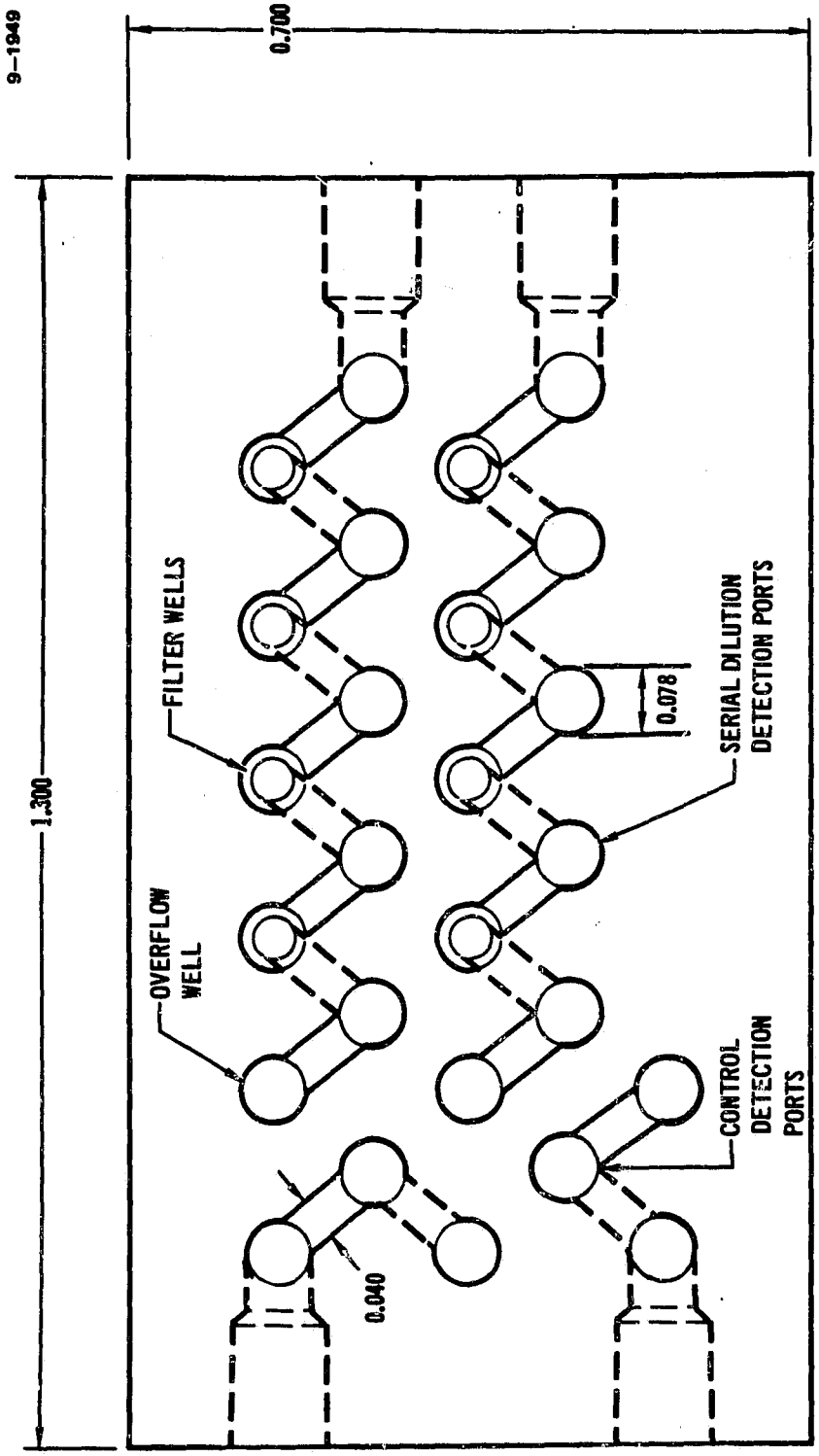


FIGURE 4-7
DUAL FILTER CASSETTE

4.2.1.2 Card - The criteria for the Card, which is the integrated cassette, were: (1) capability for multiple selective media; (2) simultaneous antibiotic sensitivity determination; and (3) simultaneous enumeration. This is achieved in the Clinical Card configuration. In addition, an Environmental Card was configured with the same well pattern but without the antibiotics or enumeration. This configuration yielded a Card which could process three samples at one time.

The design of each Card type was sent to various mold fabricators for bids and Precise Metals and Plastics of East McKeesport, Pennsylvania was selected to fabricate the mold and produce the plastic portion of the Cards. Each media well has a very slight taper (a few thousandths of an inch) to ease the Card ejection from the mold. Initial test Cards developed small stress cracks between a few wells. These did not seem to affect the initial performance results but the molder was notified and the crack problem corrected on the second run of Cards which was used for the media production run. The molded Cards are shown in Figures 4-8 and 4-9.

The measure of the Card's success was in how well it performed throughout the entire test program when used to verify the operation of the complete Microbial Load Monitor System. Many factors were inherent in its excellent operation. A few of these are: consistent tape adhesion, reliable diluent filling, and instrument detection of media changes. Adhesion of the tape is a function of the tape and its application by a taper. The adhesion was greatly improved by a change in vendors which resulted in more consistent adhesive. The loading device and related system achieves a reliable fill when used with a high quality vacuum pump. Evacuating both the inside and outside of the Card is the principal reason for its excellent fill without bothersome bubbles. This method of filling is also instrumental in minimizing stress produced tape bowing and optical path changes which might hamper the instrument's detection capabilities. Reliable results are achieved in the final end product (packaged and sealed Cards) when conscientious work in producing the Cards is performed.

The results of this portion of the contract is two Card versions; a Clinical Card capable of testing an inoculum against 12 selective media, determining antibiotic sensitivity for 11 organisms, and simultaneously determining a relative

9–1626

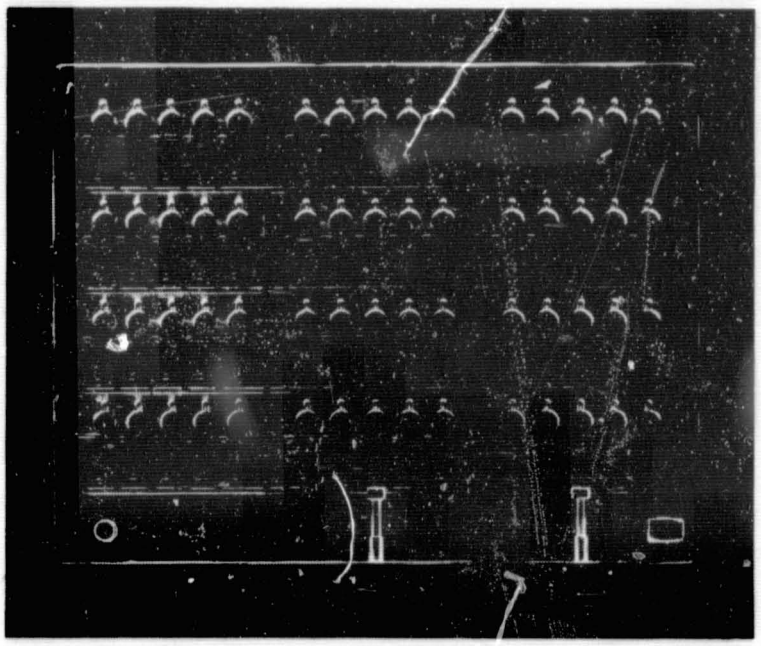


FIGURE 4–8
MOLDED CLINICAL CARD

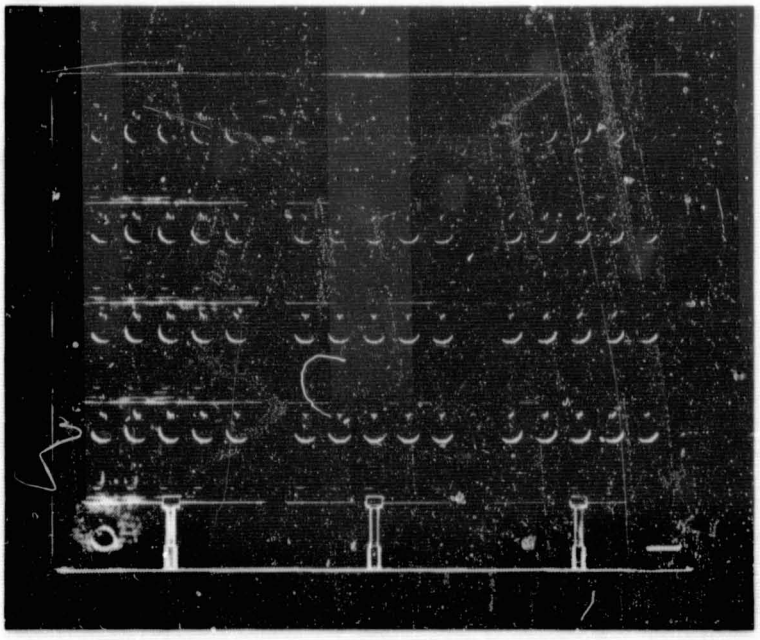


FIGURE 4–9
MOLDED ENVIRONMENTAL CARD

number of organisms for enumeration; and an Environmental Card capable of testing three inoculums simultaneously against 13 media.

4.2.2 Sample Loading Equipment Results - Studies and tests conducted for loading samples into the cassette and Card resulted in the fabrication and extensive use of two different Sample Loading Systems. The first was developed for Earth-based loading of samples into the cassette. The second was developed for use under null-g conditions.

4.2.2.1 Earth-Based Loading Equipment - For loading cassettes in a one-g environment, the multistation filling system described in Section 3.2.2 was adequate. This was demonstrated by the many tests performed in cassettes which were loaded by this station. The multistation concept has a characteristic of station interaction if individual stations are switched to evacuate at different times. The succeeding stations will slightly compromise the vacuum of previous stations with a burst of air. This will result in longer evacuation times to achieve reliable fills. The extension of evacuation time depends greatly on the quality of vacuum system used and the rate in which it can pump.

Tests conducted with this system showed that a five minute evacuation time at 5mm Hg vacuum level and a medium filling rate (approximately 0.08 ml/sec) would achieve the most reliable fill. The vacuum level was achieved with a good quality vacuum pump (bench systems are usually not adequate). The evacuation time was determined by the operator's watch and the filling rate was a system adjustment.

Two deficiencies became apparent with this method of loading cassettes. Excellent seals on all loading device joints were required to be able to achieve a low internal vacuum, and the combination of low internal cassette vacuum and high external atmospheric pressure caused stretching and distortion of the tape surface over each well. Both deficiencies can be alleviated by enclosing the entire loading device and cassette in a vacuum chamber. This was a major characteristic of all designs for use under null-g.

4.2.2.2 Null-G Sample Loading System - The Sample Loading System Concept C Flight Prototype as described in Section 3.2.2.4 was fabricated, tested and used for biological tests of the Clinical and Environmental Cards. Requests for bids

were sent for mold fabricators and Precise Metals and Plastics of East McKeesport, Pennsylvania was selected. Some small details were changed to enable efficient mold produced parts such as a slight taper to long draws (solid sample port). The Sample Receiving and Card Loading Device (SRCLD) plastic parts were molded by the thousands. The SRCLD with annotated parts is shown in Figure 4-10.

During the engineering test and the initial biological tests, five problem areas concerning the SRCLD were identified.

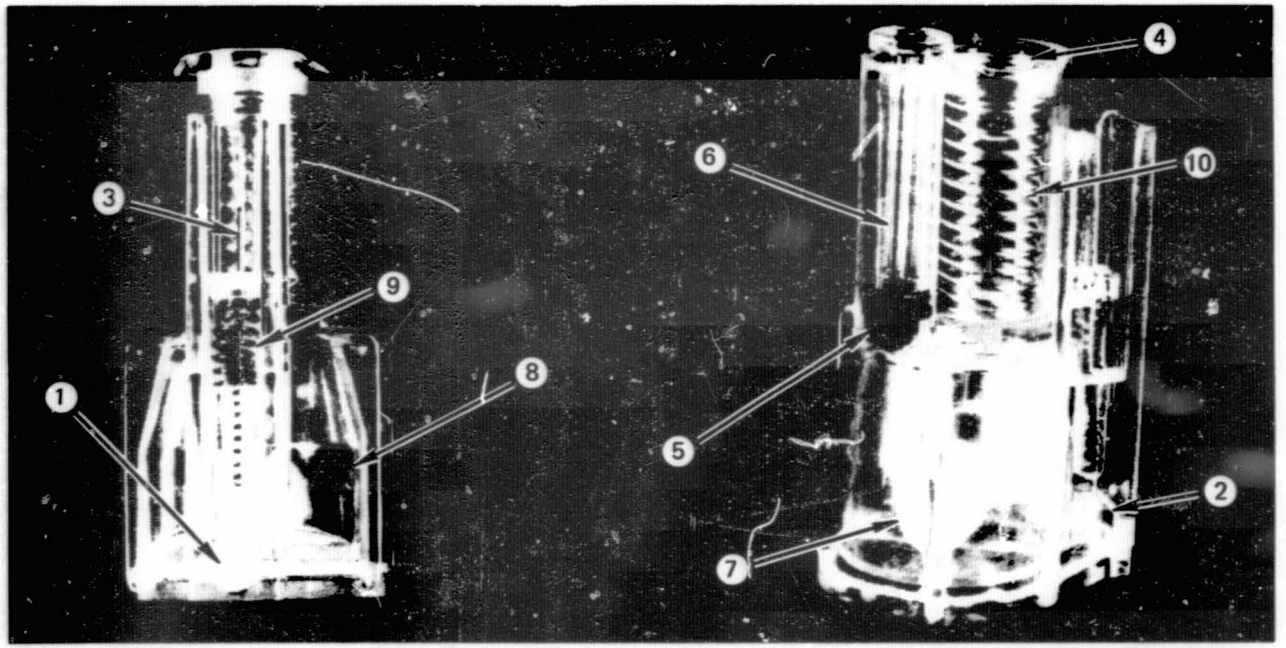
1. The double ended filling needle was too short to completely pass through the Card septum if the length of the Card septum was not closely controlled. Action taken was to respecify the double ended needle by increasing its length and thus relieving the close tolerance on the Card septum.

2. Isolated cases of filling needle jamming near the Card loading septum were traced to improper drilling of a retaining pin hole which is drilled by the producer of the plastic parts. He was notified and inspection now includes this item.

3. Improper Card fill due to septum material clogging needles. Action taken was to have all needles with an anticoring bent tip. In addition, the Card filling septum area of the SRCLD was found to have too small an opening for the needle. It subsequently was drilled to an enlarged diameter of 0.062 inch after the septum area has been filled and cured. The drilling cleared the entrance hole of excess septum material.

4. Improper fill due to magnet driven impellor not rotating at the moment of fill. Actions taken were: parts inspection and quality fabrication; the solid sample plunger was spaced to remove the rubber tip from the impellor chamber; replaced original driver magnets with more powerful and better designed ones (see Table 4-34, Magnet Driver Analysis); and modified the software for the SLS to allow more operator action in rotation checks. Of the 53 with rotational difficulties in the table, only four resulted in bad fills.

9–1762



1. Liquid Sample Port and Septum
2. Card Loading Septum
3. Card Filling Needle
4. Evacuation Plunger
5. Solid Sample Port
6. Solid Sample Plunger
7. Magnetic Impeller
8. Mixing Chamber
9. Card Filling Return Spring
10. Evacuation Return Spring

FIGURE 4–10
SAMPLE RECEIVING AND CARD LOADING DEVICE

TABLE 4–34
DRIVER MAGNET ANALYSIS

9–1945

MAGNET DRIVER CONFIGURATION	TYPE CARD	SCRLD'S TESTED TOTAL	TOTAL NO. OF SRCLD'S WITH ROTATION PROBLEMS AND PERCENTAGE	POSITION OF SRCLD'S & NO. WITH ROTATION PROBLEMS AND PERCENTAGE		
				LEFT	CENTER	RIGHT
ORIGINAL	CLINICAL	58	26 (44.8%)	–	9 (15.5%)	17 (29.3%)
NEW MAGNET DRIVER IN RIGHT POSITION.	CLINICAL	110	22 (20%)	–	15 (13.6%)	7 (6%)
NEW MAGNET DRIVER IN ALL POSITIONS	CLINICAL	16	0	–	0	0
	ENVIRONMENTAL	52	5 (9.6%)	2 (3.8%)	1 (1.9%)	2 (3.8%)

5. Leaky SRCLDs due to incomplete ultrasonic weld on bottom piece. Action taken required critical alignment of pieces during ultrasonic welding and using a clean hot soldering iron on any leaks resulting from leak checks. In a test sample, critical alignment resulted in an 80% increase in good units.

The vacuum chamber has operated reliably throughout the test program after initial engineering checkout and test. Time and vacuum checks are made during loading as described in Section 4.2.5, Software Results.

4.2.3 MLM Incubation and Detection Instrument - There were two principal instruments developed during this contract, the MLM-S (Semi-automated sequential sampling) with its related carousel and the MLM System Flight Prototype. Both units incorporated digital logic and analog multiplexing for increased reliability and ease of use.

4.2.3.1 MLM-S and High Volume Carousel - The MLM-S and its associated high volume carousel was a great improvement over the four/five channel MLM. The MLM-S was designed for 10 cassettes with 10 channels each. The carousel improved this to a capability of 150 cassettes. The major improvement was really not in the number of cassettes it could handle, but in the addition of a programmable calculator to monitor the readings, gather data and provide time history profiles at the end of the microbiological tests. The stability of the electronic instrumentation was

within two percent excluding random data excursions attributable to noise (voltage or current) spikes affecting preamps and analog to digital convertors.

The MLM-S was fairly tedious to completely set up for operation. Adjustment of the light emitted from each LED was made by a potentiometer. One hundred channels equaled 100 potentiometer adjustments. Temperature checks could not be made during a run and had to be made with external instrumentation. These and other items were considered when designing the MLM System Flight Prototype.

4.2.3.2 MLM System Flight Prototype - The development of a Microbial Load Monitor which would operate in null-g but would also operate under one-g conditions resulted in the MLM System Flight Prototype as described previously in Section 3.2.3.2. It was designed (and has been operated) as a completely integrated unit. Some of the results of this design are stated in the following paragraphs.

This instrument is microprocessor controlled rather than being externally monitored. This results in an instrument which is simpler to operate. Through the use of microprocessor software, the operator is prevented from performing most error causing actions. Through the use of an alphanumeric keyboard and display, the microprocessor accepts or rejects commands. On items which are not instrumented, the operator may be asked to visually verify before continuing program actions.

Controlled by preprogrammed instructions, the microprocessor can perform the following instrumented functions.

a. Calibrate each Incubating/Reading head in less than one minute when commanded (and notify if in error).

b. Initiate head reading when commanded (each head separately) and continue at preset intervals (presently 30 minutes) for a predetermined length of time (presently 13 hours).

c. Read a 24-hour clock (month, day, hour, and minute) and set to input time when commanded.

d. Monitor each separate head temperature and notify if incorrect.

e. Perform real time calculations to determine changes in light transmitted by each well.

f. Store and retrieve data on magnetic tape.

g. Let operator know intermediate results (0 to 13 hours) when requested without opening incubator.

h. Print final results after 13 hours when requested.

The power supplies presently used in the flight prototype unit limits its use to 60 hertz, 120 volt primary power. Table 4-35 details the maximum d.c. power requirements for the five incubator/reader head instrument. These values are listed to show approximately what will be needed if the power supplies are changed to d.c. to d.c. convertors envisioned for Spacelab use. The regulated supplies are +5 volts d.c. (26.2 watts), -15 volts d.c. (20.1 watts), and +15 volts d.c. (2.7 watts).

The power supplies can accommodate either a constant high or low line voltage (130 volts high or 110 volts low). This is accomplished by changing leads on the power supply transformer.

TABLE 4–35
D.C. POWER REQUIREMENTS
FIVE HEAD CONFIGURATION

9–2061

TYPE	SUPPLY OUTPUT	SUPPLY INPUT	EFFICIENCY
REGULATED	49 WATTS	159 WATTS	31%
UNREGULATED (PEAK)	110 WATTS	137 WATTS	88%
UNREGULATED (AFTER WARMUP)	45 WATTS	57 WATTS	79%

NOTE: ALL SUPPLIES 60 HERTZ 120 VAC INPUT

4.2.3.2.1 Microprocessor and Memory - During engineering design and development the IMP-16C microprocessor had 4K of CMOS read/write memory and 1K of PROM available. After the biological testing was accomplished, this was changed to an 8K EPROM board (replaced one 2K CMOS memory board) and 2K of CMOS read/write memory. This change enables all programs to reside in memory for operational use without the lengthy paper tape readings used during the development phase. It also makes the memory less volatile.

National Semiconductor MM5204 EPROMs are used and each contain 512 one byte portions of a sixteen bit (two bytes) word. Programming of the EPROM was accomplished on an IMP-16P development system with a programming board capable of programming either MM5203s or MM5204s.

No major problems occurred with either microprocessor or memory during biological testing. One isolated instance of an EPROM failure occurred. The program would not operate correctly but when read from the front keyboard all instructions were correct. Replacing the MM5203 EPROM used at that time corrected the problem. The EPROM was discarded as no reason could be found for its failure during the execution of one memory location (instruction) and its otherwise proper reading unless the integrated circuit was defective. Reprogramming the same device did not correct the problem.

4.2.3.2.2 Incubating/Reading Head - Testing of the first Incubating/Reading Head revealed only one problem. The LED connections in the arrays were reversed (cathode and anode). This was corrected by rewiring the emitter board in the first head and then redesigning the emitter board for additional heads. Additional assembled heads revealed a few heads with an electrical instability in the detector electronics. The original design used a voltage reference bypassed (paralleled) with a 1μf capacitor. Subsequent investigation revealed the reference (an Analog Devices AD580) would oscillate with a capacitive load of between 0.68μf and 2μf. A telephone conversation with the manufacturer's application engineer revealed that the AD580 was capacitive load sensitive. This capacitor was removed from all detector boards and the heads retested for stability. The stability test results showed no oscillatory instability in any head. In addition, more stable values were seen on heads which previously did not reveal an oscillation (could not be seen on an oscilloscope).

Performance of the incubating/reading heads during biological testing has been very good with no failures during biological tests. However, some annoying difficulties did occur during fabrication and checkout of the heads. Phototransistor dies when bonded to the ceramic substrate and wire bonded to connector pins and metalization wires would test good, but after cover glass mounting they would fail. These failed units would have to be removed, replaced and checked again. Once units have operated for a few hours the spontaneous failure rate is very low, only about eight units out of 800. Other failures have occurred due to mishandling - cracked or loose cover glass, loose connector pins, and loose ceramic substrate in the plastic housing.

4.2.4 Ancillary Equipment Results - This section is limited to the performance of equipment needed to produce loading devices and Cards now in use. Previously used ancillary equipment was described in Section 3.2.4 and comments made on its performance.

4.2.4.1 Card Preparation Equipment Performance - The performance of the ancillary equipment in preparing Clinical and Environmental Cards for delivery was quite good. Nineteen wrapped trays containing a total of 642 cleaned Clinical Cards were used to produce 300 Cards for delivery and a sufficient quantity of control Cards for testing. Loading media into the Cards required approximately 7-1/2 hours of preparation, loading and clean-up time. Four people were occupied during the majority of this time. The Cards had been previously prepared by cleaning and taping the bottom with the Taper which was described in Section 3.2.4.1. This operation was performed on a laminar flow bench.

Loading the cleaned Cards began by preparing the Media Pumping System shown in Figure 4-11. The procedure for preparing the system has been previously described in Section 3.2.4.2. A laminar flow bench is also used to load media into the Cards. With the Media Pumping System primed, the media reservoir is inserted into the Dispensing Station, as shown in Figure 4-12. Each media reservoir was used for approximately 40 Cards before being replaced and refilled.

The dispensing head is lowered and media is sucked from the reservoir into the needles, tubing and syringes. The Card and Card holder is then inserted into the dispensing station as shown in Figure 4-13, and the dispensing head is lowered.

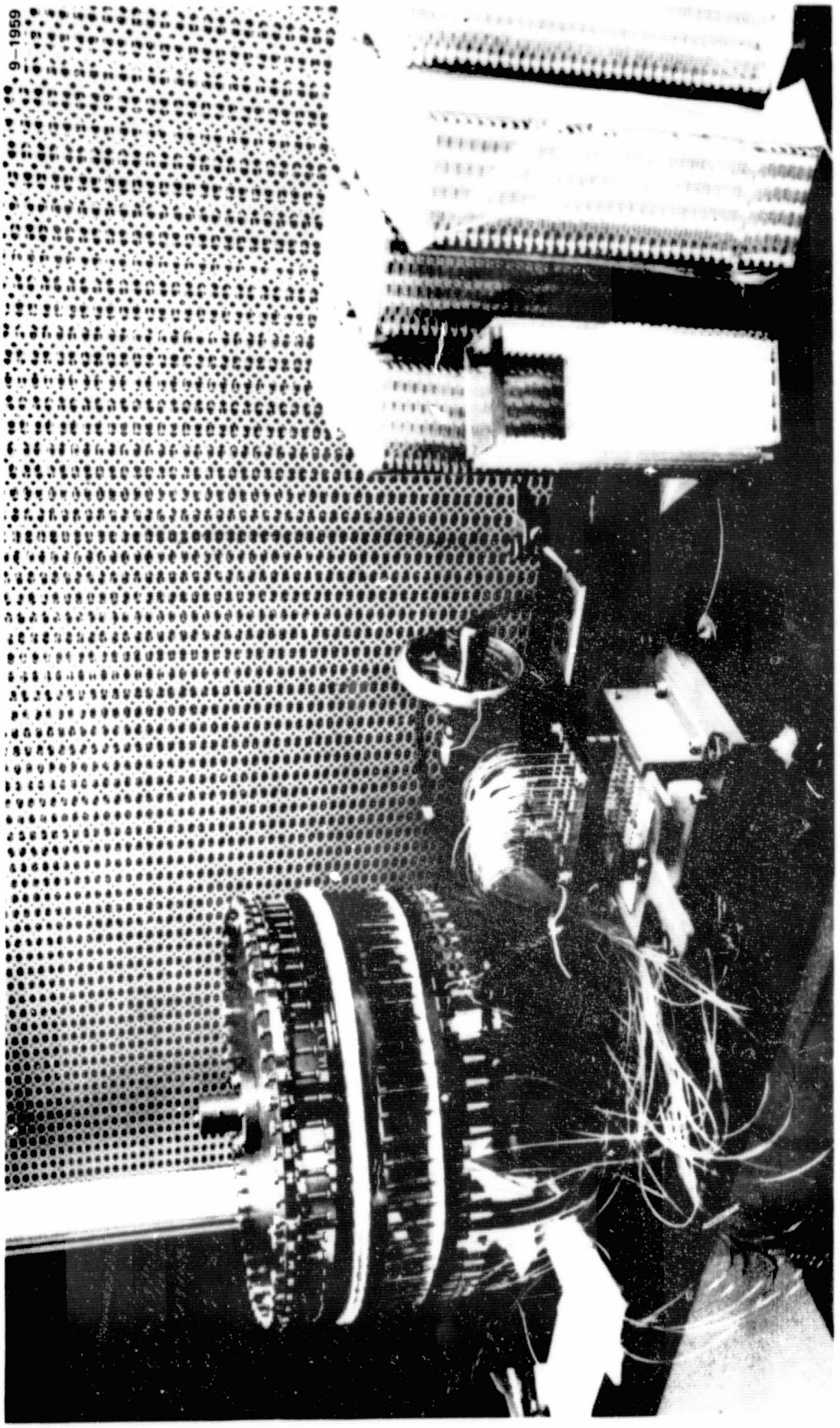


FIGURE 4-11
MEDIA PUMPING SYSTEM

FIGURE 4-12
DISPENSING STATION WITH MEDIA RESERVOIR

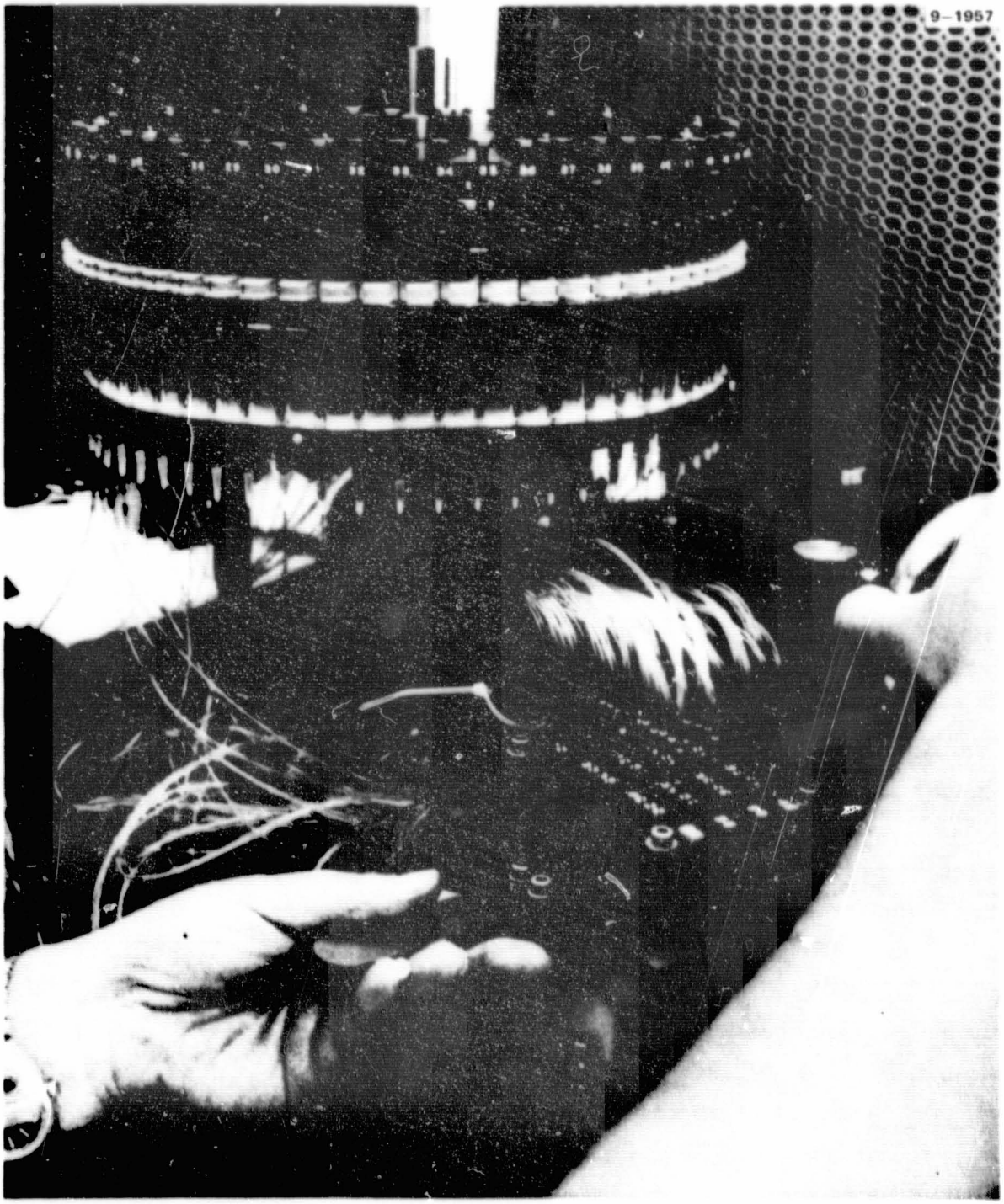


FIGURE 4-13
DISPENSING STATION – CARD LOADING

This activates the microswitches which initiate the pumping of media into each Card well. The dispensing head is raised and the Card holder and Card are removed, Figure 4-14. The Card is then removed from the Card Holder and the Card placed into a numbered Card tray (Figure 4-15). When the tray is full it is placed in a low temperature freezer, which is maintained below -40°C. The frozen Cards are now ready for freeze drying, final taping and packaging in plastic lined aluminum foil pouches.

The final equipment setup is shown in Figure 4-16. The freeze dryer door and Card taper are completely enclosed. The enclosure is flushed with dry N_2 to remove moisture laden air from the enclosed space. The nitrogen connection is shown at the lower left hand corner of the plastic enclosure around the taper. When repressurizing the freeze dryer the nitrogen is switched to its air inlet so that the chamber is back filled with dry nitrogen when the freeze drying vacuum is released. It is important to remove the Cards from the freeze dryer and tape them in a dry environment so that the lyophilized media will not rehydrate.

Arm holes, as shown in Figure 4-17, allow removal of the Card trays from the freeze dryer and loading of the Cards into the Card reservoir of the Taper. Arm holes on the opposite side, Figure 4-18, allow operation of the Taper and production of taped Cards. Cards are more easily cut after they exit the plastic enclosure. This is shown in both Figures 4-17 and 4-18.

A bottom view of the complete freeze dried and taped Clinical Card is shown in Figure 4-19. Cards from the taping operation are then pouched and heat sealed as shown in Figure 4-20. Cards were examined after taping and were segregated according to the following categories - NASA delivery, quality control and other tests, and rejects. Cards were rejected for the following reasons: (1) substantial media rehydration, (2) splashed media or foreign matter on the Card surface, and (3) missing or insufficient media in any well. Cards saved for MDAC Quality Control or other tests had very few of the above problems. Cards selected for NASA delivery had no visible defects at the time of pouching and sealing. The results of these operations for the Clinical Cards are shown in Table 4-36. The high number of rejects the first day was due to overloading the freeze dryer. Thereafter only four or fewer trays were processed at one time.

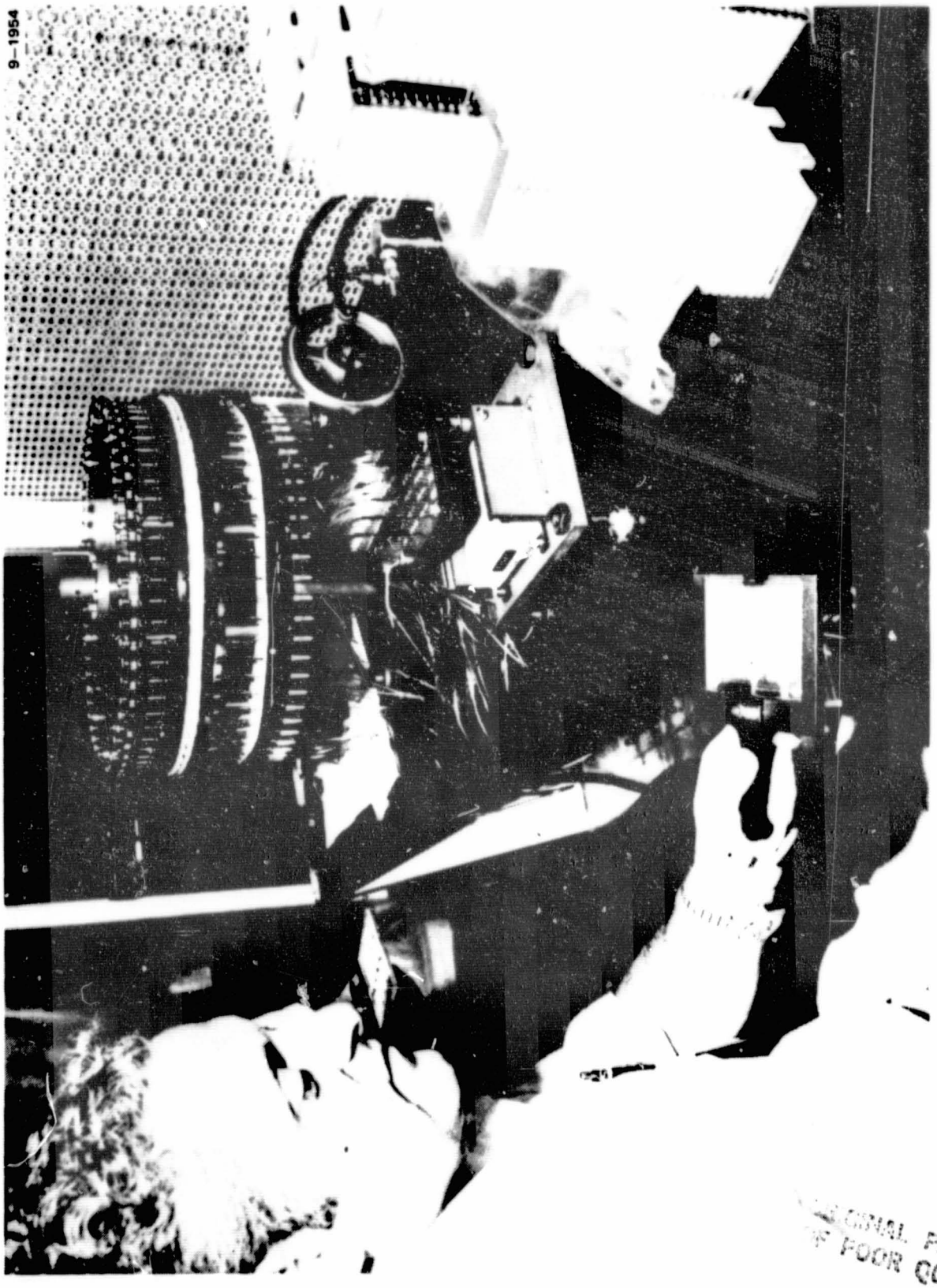


FIGURE 4-14
CARD HOLDER WITH LOADED CARD

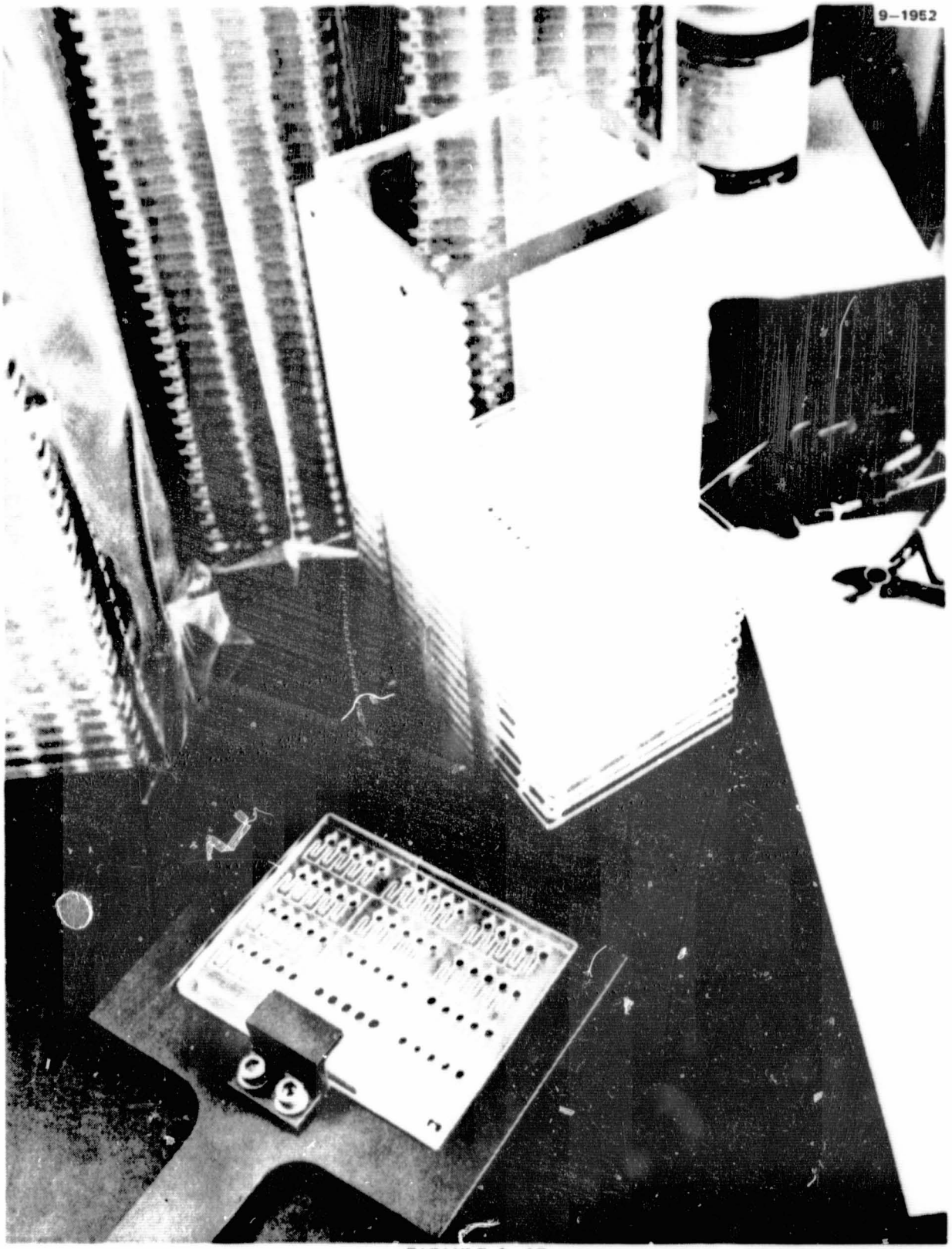
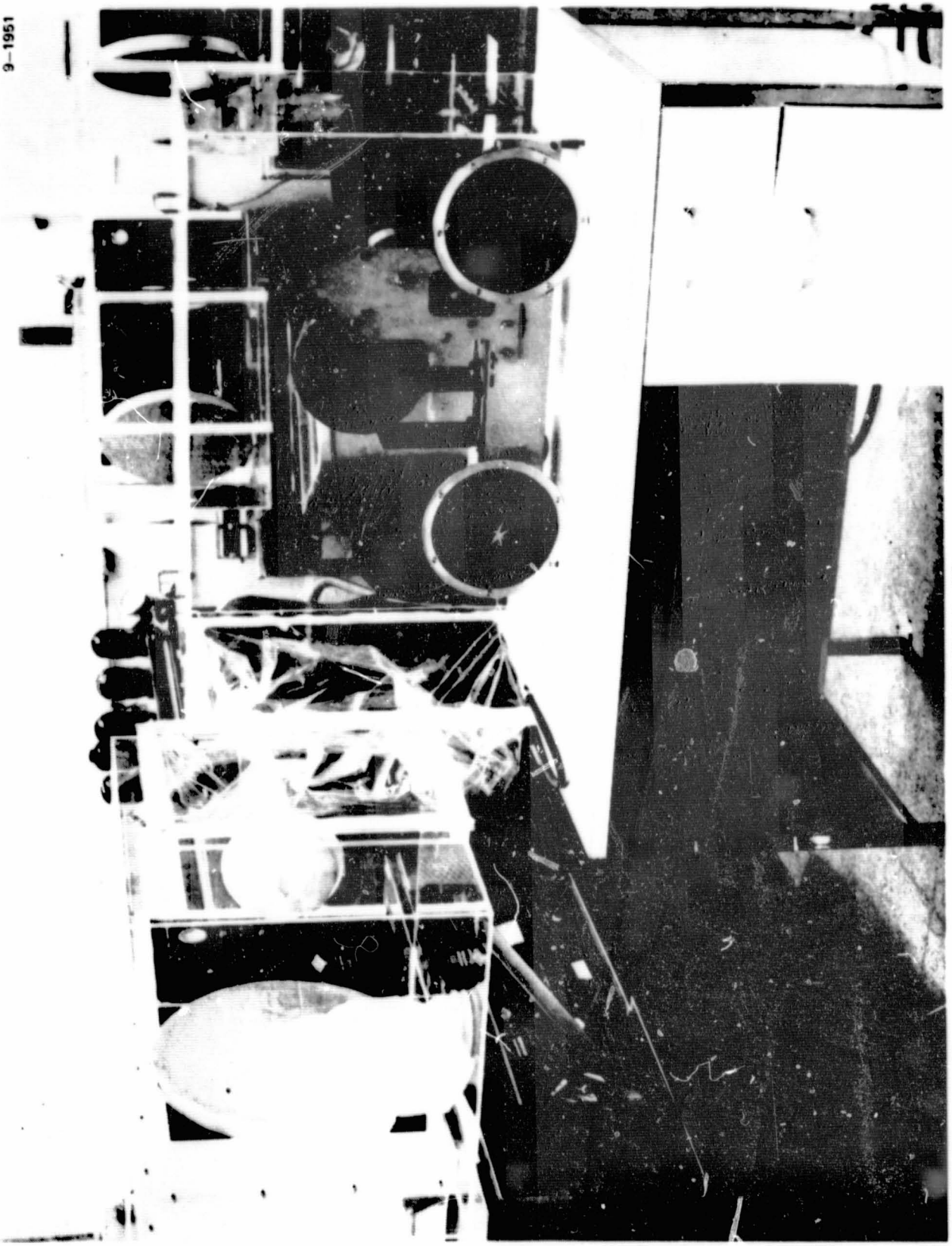


FIGURE 4–15
CARD TRAYS



9-1951

FIGURE 4-16
CARD FINAL TAPING EQUIPMENT

9-1953

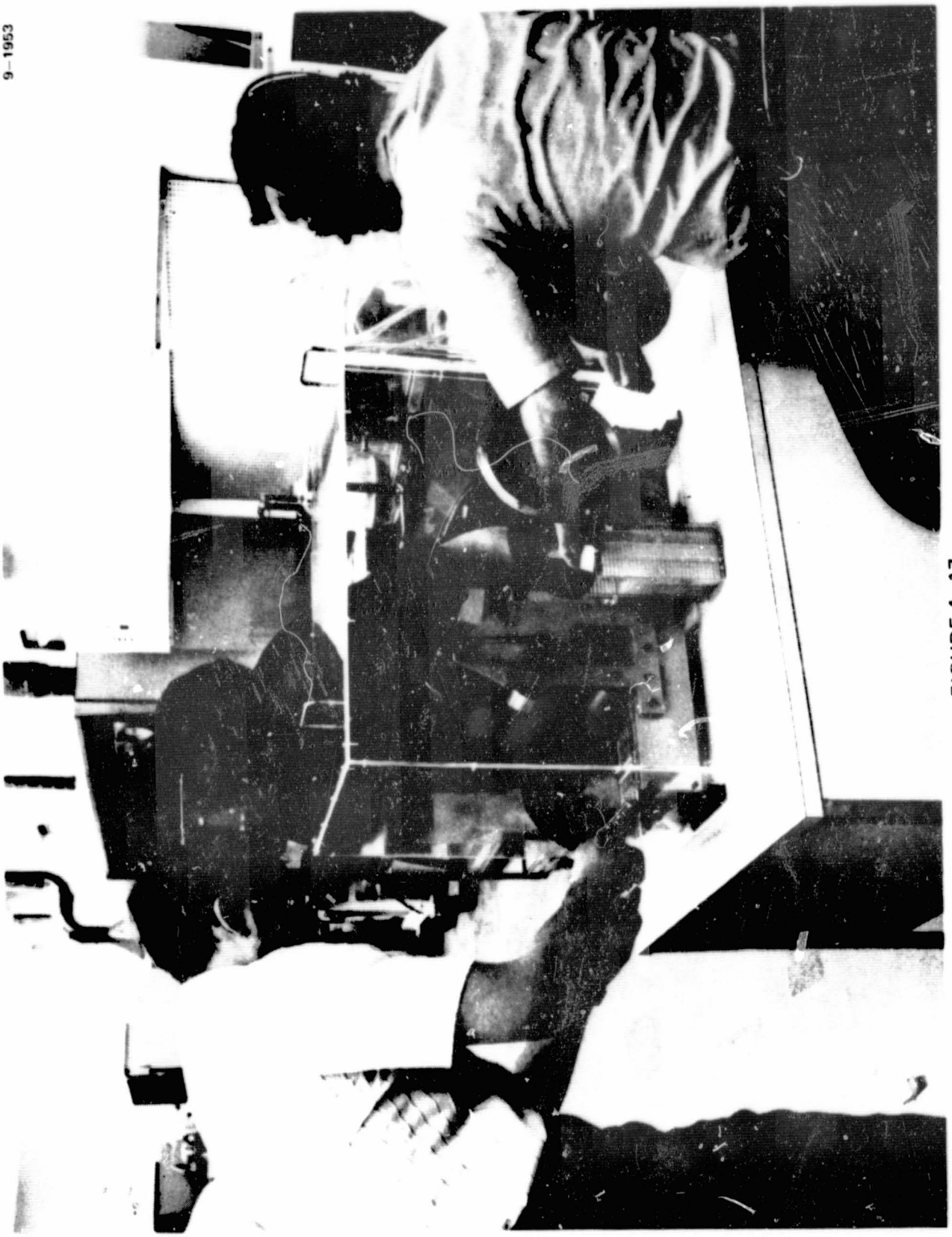


FIGURE 4-17
TAPER LOADING AND TAPING

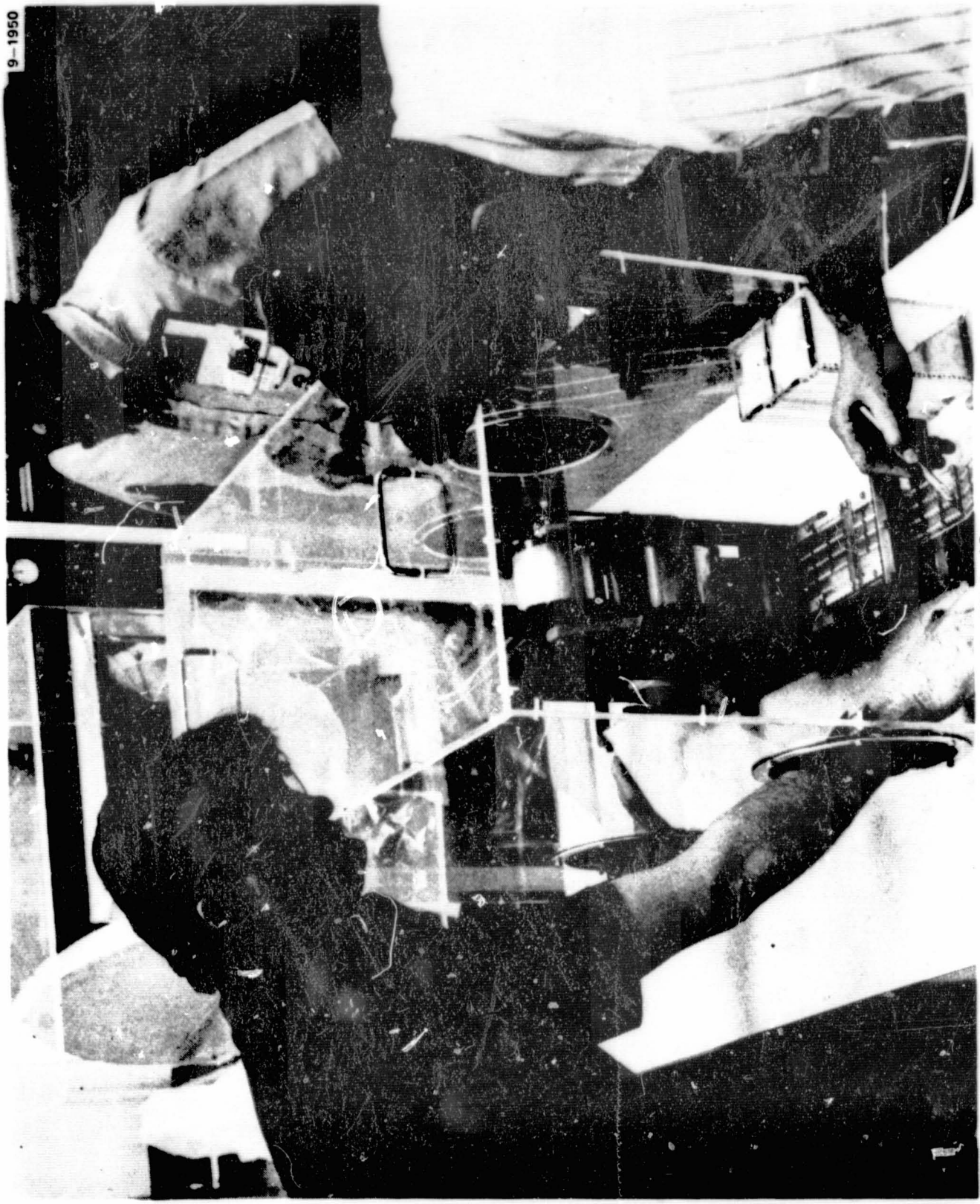


FIGURE 4-18
TAPER OPERATION

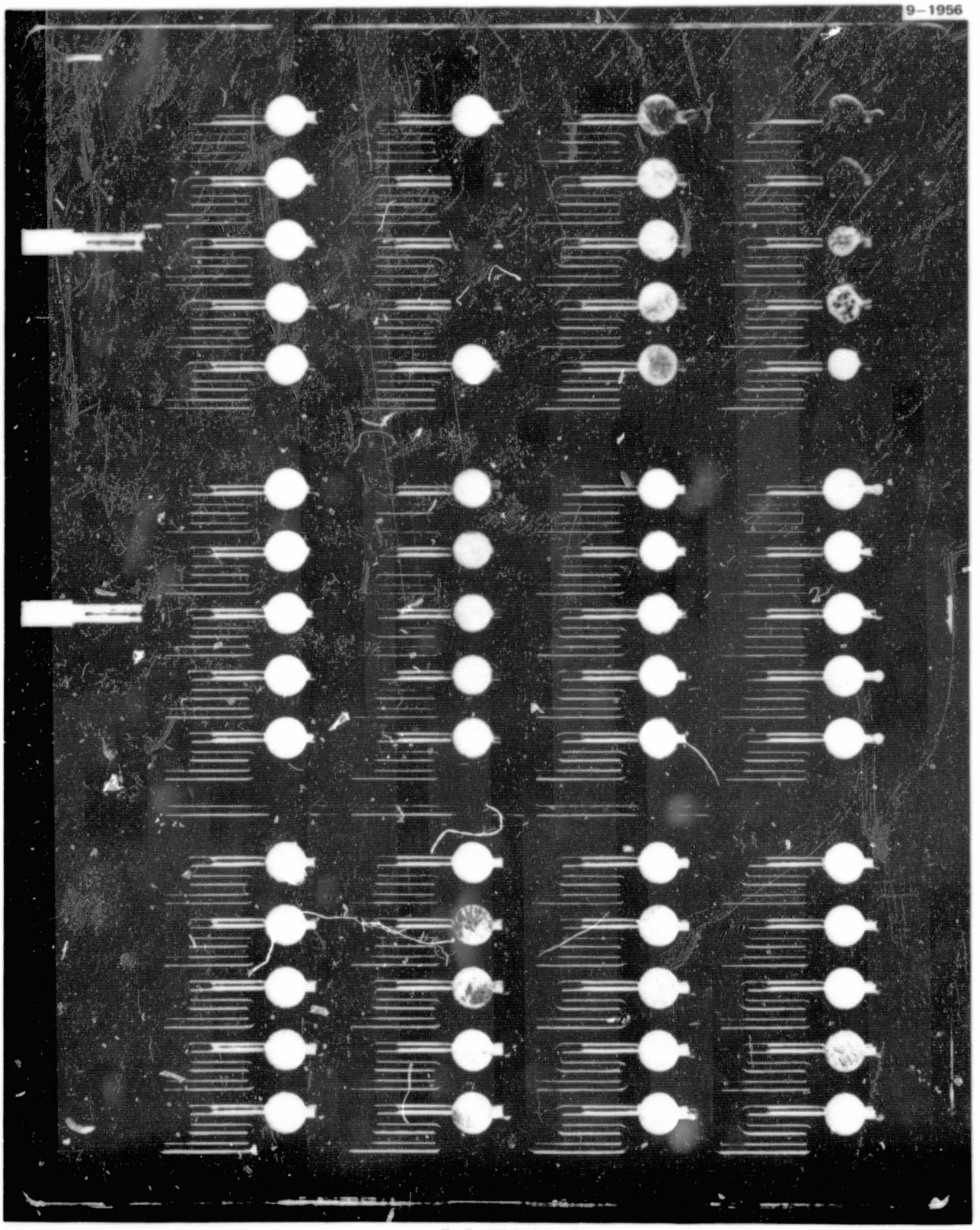


FIGURE 4-19
COMPLETED CLINICAL CARD, BOTTOM VIEW

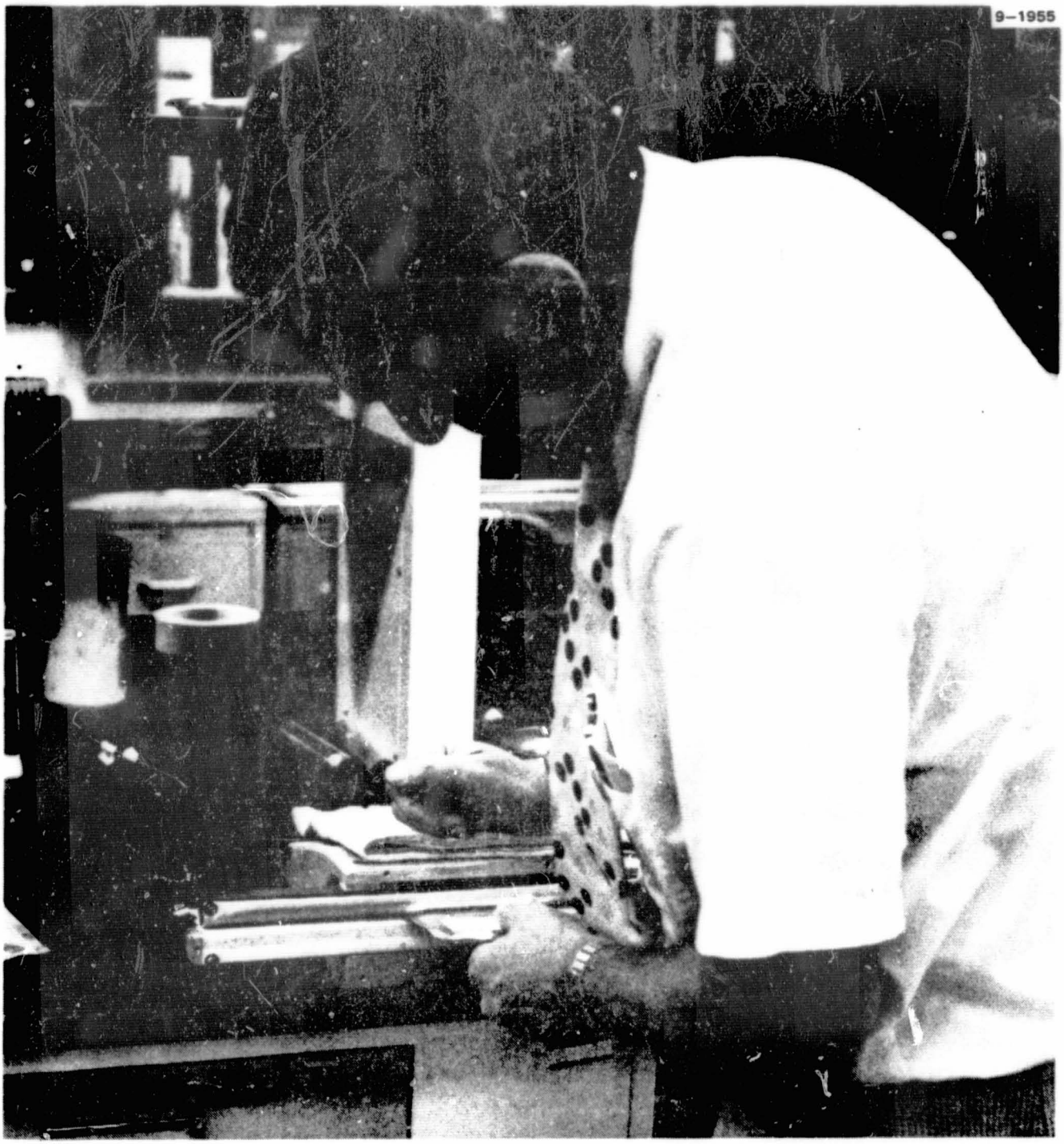


FIGURE 4–20
CARD POUCHING AND SEALING

TABLE 4–36
CLINICAL CARD FREEZE DRYING RESULTS
9–1946

DAY	1	2	3	4	TOTAL
NASA DELIVERY	75	85	96	63	319
QUALITY CONTROL	45	23	43	18	129
REJECTS	39	12	5	27	83
TOTAL	159	120	144	108	531

Environmental Cards were prepared similar to the Clinical Card preparation. The first attempt at producing these Cards was aborted when the vacuum pump belt of the freeze dryer broke. The loss of vacuum during the freeze drying cycle ruined the Cards. A second run was successfully completed from which 56 Cards were selected for NASA delivery, 37 Cards selected for MDAC Test, and 10 Cards rejected.

4.2.4.2 SRCLD Ancillary Equipment - With the large amount of hand labor required in preparation of SRCLDs, the effectiveness of any ancillary equipment depends, to a large extent, on the care with which a person uses it. An example is the sonic welding of SRCLDs.

Table 4-37 lists the results of an ultrasonic welding test. Two methods were chosen. One used normal care in aligning the SRCLD body with the bottom piece. The second used extreme care in aligning the pieces, i.e., near perfect alignment was attempted. Normal care had less than 50% good parts while extreme care achieved

TABLE 4–37
SRCLD WELDING
9–1943

WELDING TECHNIQUE	QTY TESTED	VISUAL REJECT	PRESSURE CHECK			ACCEPTABLE UNIT	% ACCEPTABLE UNIT
			SEPTUM FAILURE	LARGE LEAK	SMALL LEAK		
NORMAL	47	8	2	2	12	22	47
EXTREME CARE & ALIGNMENT	24	0	0	2	0	22	92

over 90% good parts. This test showed that alignment of bottom and body pieces of the SRCLD will have a large effect on the effectiveness of the seal. An undetermined effect is the stress on the weld due to electron beam sterilization since device testing is accomplished before sterilization.

4.2.5 Software Results - Software development for the Microbial Load Monitor System has resulted in a complete program capable of operating the MLM electronics with minimal operator supervision. The MLM system will monitor changes in the preprogrammed Cards and make decisions concerning organism type, antibiotic sensitivity and enumeration.

The software has been designed to require minimal operator training. The commands, as given in Table 3-7 of Section 3.2.5.2, are four letter abbreviated explanations of what the operator is requesting the MLM to perform. Most routines have logical steps or checks by which the microprocessor verifies proper operation of equipment. For example, after either an EXIT or PLOT routine has been used, a READ command will result in a "DATA INVALID" message on the display and "END OF HEAD READING DUE TO CAL AND/OR TEMPERATURE ERROR" will be printed on the Teletype. This same Teletype message is used when the temperature of an active head is lower than 33.5°C or higher than 37.5°C. The "DATA INVALID" message is also displayed when requesting STAT on an inactive head.

The software and MLM instrument with the Sample Loading System have performed quite well together during the many months of gathering data for setting and verifying threshold data of each media, or antibiotic and media combination. Performance of the software is best described by a quick description of one Card as it is loaded, incubated and the results retreived by the operator. The assumptions made in the description is that the MLM instrument has been unplugged from 60 hertz power for sufficient time for the incubating reading heads to cool to room temperature and that a Clinical Card is to be used. Operator commands are underlined and MLM responses are placed in quotes in the description.

The MLM is plugged into the 60 hertz, 120 volt outlet. The alphanumeric display likely will have random characters until the internal clock is reinitialized by the power up routine and then it will display the message "POWER FAILED," and a message printed on the Teletype if the Teletype is in the line mode.

At least 15 minutes should be allowed for head warm-up. During this time the vacuum pump is turned on. During the warm-up time the display will flash "TEMP ERROR #N," where "N" is a head number. Commands can still be given by waiting until the display shows the first character of the command and then keying the other three characters.

Since the internal clock will be incorrect (by the amount of time the power was off) it is initialized with the commands IDAT and ITIM, which request numerical information (two digits each) with leading zeros for numbers less than ten. A fresh digital quality mangetic tape cassette (with no clear leader) is inserted into the magnetic tape recorder, and the record and play levers pressed simultaneously.

The head is calibrated after the head temperature has stabilized. The display will no longer flash "TEMP ERROR #N" if the temperature is within ±1°C of the preset nominal value of 35.5°C. A more stable calibration is performed if the temperature is within a few tenths of a degree of the steady nominal. The command TEMP "#"N allows the operator to check the temperature on head N. Calibration is initiated by the command CALH "#"N where "#" is a response by the MLM and N is the selected head (1 through 5). At the end of calibration, the internally stored calibration values are printed on the Teletype and stored as a calibration record on the tape recorder. If the magnetic tape recorder had not been record enabled, the display would flash "PRESS RECORD" until the levers were correct and any key on the keyboard depressed to stop the flashing.

A Card to be loaded from prepared SRCLDs is placed in the loading chamber as described in the operator's manual. The command LOAD is given through the keyboard. The MLM spins up the driver magnets and asks: "IS SPEED OK?" The operator checks for rotation of the magnets in all SRCLDs. Answering with Y means yes, continue; D means lower speed and try again, etc., as described in the operator's manual. Vacuum is checked and a running display given of evacuation time and pressure. At six minutes the message "IS SPEED OK?" is displayed. Verification of magnet rotation is again performed. Upon answering Y, the Card and SRCLDs are joined and the Card begins to fill as the vacuum is released. The display now shows the pressure reading as it returns to atmospheric pressure.

When loading is finished the display reads "LOADED" and the Card may be removed from the loading chamber. The Card is allowed to stabilize at room temperature for approximately seven minutes and in the Incubating/Reading Head for another seven or eight minutes. Card reading is initiated by commanding READ"#"N as in the above command CALH"#"N. The MLM will request "CARD TYPE" and the character C (for clinical) is keyed.

Initial readings of all 60 channels are taken and stored in memory, printed on the Teletype and recorded on the magnetic tape recorder. The reading cycle repeats at intervals of 30 minutes and computations on present versus initial readings are made and compared to threshold values. Note is made of those that exceed the preestablished threshold and what time it occurred. Interim results are requested anytime between 0 and 13 hours (by the command STAT and appropriate head number) and the MLM responds on the display with a list of abbreviations of positive media or "NO POSITIVES." A request related to an Environmental Card will also request which "SECTION?". The sections are Left A, Center B and Right C.

Complete results can only be requested after 13 hours or the display reads: "INCU NOT COMPL." After 13 hours, a request of FINS (and appropriate head number) will result in a listing on the Teletype of all positive media and related antibiotic results, time to reach positive for each media and an enumeration value. Date, time and head number are also printed.

If Time History Profiles are needed the magnetic tape should be rewound and the recorder enabled for play only. The command PLOT"#"N can now be given. The magnetic tape is searched for all 13 hours of data (27 points as counted on the display), resorted and plotted as Time History Profiles in groups of five as shown in the examples of Figure 4-21. The abbreviations used are those listed in Tables 3-8 or 3-9 of Section 3.2.5.2.

The examples are two plots out of twelve plotted for a Clinical Card. Heading information details the incubation head from which the data was taken, the plot symbols (O, X, +, #, *) and their associated channel and selective media or antibiotic, and the date and time at which the test was started. The percentage change is a decrease in transmitted light. Plotting identical valued points is accomplished by using the numbers 1 and 2. All symbols within the parentheses are

MICROBIAL LOAD MONITOR

```
HEAD #3                                    HEAD #3                                    9-1944
0=CH# 01   MEDIA  EC                       0=CH# 56   MEDIA  EN
X=CH# 02   ANTIB  FD                       X=CH# 57   ANTIB  EN
+=CH# 03   ANTIB  SX                       +=CH# 58   ANTIB  EN
#=CH# 04   ANTIB  NA                       #=CH# 59   ANTIB  EN
*=CH# 05   ANTIB  AM                       *=CH# 60   ANTIB  EN

DATE 0613    START TIME  1012            DATE 0613    START TIME  1012

                PERCENTAGE CHANGE                          PERCENTAGE CHANGE
          0  16  32  48  64  80  96                     0  16  32  48  64  80  96
        0 1  :   :   :   :   :   :  (0X+#*)          0 1  :   :   :   :   :   :  (0X+#*)
          1  :   :   :   :   :   :  (0X+#*)            1  :   :   :   :   :   :  (0X+#*)
E       1 1  :   :   :   :   :   :  (0X+#*)        E   1 1  :   :   :   :   :   :  (0X+#*)
          1#*0:   :   :   :   :   :  (X+)              1  :   :   :   :   :   :  (0X+#*)
L       2 1 # : 2 :   :   :   :   :  (X+)0*        L   2 1  :   :   :   :   :   :  (0X+#*)
          1 # :   :*  :  0:   :   :  (X+)               1  :   :   :   :   :   :  (0X+#*)
A       3 1 # :   :  *:    :0   :  (X+)            A   3 1  :   :   :   :   :   :  (0X+#*)
          1  #:   :   :*  : 0  :  (X+)                 12  :   :   :   :   :   :  (0X+)#*
P       4 1  #:   :   : * : 0  :  (X+)            P   4 : 1 *  :   :   :   :   :  (0X+#)
          1  #:   :   :   *: 0  :  (X+)                :   :  1X*  :   :   :   :  (0+#)
S       5 1  #:   :   :   *: 0  :  (X+)            S   5 :   :  102 :   :   :   :  (+#)X*
          1  #:   :   :   *: 0  :  (X+)                :   :  :10X*  :   :   :   :  (+#)
E       6 1  #:   :   :   *:  0:  (X+)            E   6 :   :   : 10X*  :   :   :  (+#)
          1  #:   :   :   *:  0:  (X+)                :   :   : 10X*  :   :   :  (+#)
D       7 1   #   :   : * :  0:  (X+)            D   7 :   :   : 10:2  :   :   :  (+#)X*
          1   #   :   : * :  0:  (X+)                :   :   : 10:2  :   :   :  (+#)X*
        8 1   #   :   : * :  0:  (X+)              8 :   :   : 10X*  :   :   :  (+#)
          1   #   :   : * :  0:  (X+)                :   :   : 10X*  :   :   :  (+#)
T       9 1   #   :   : * :  0:  (X+)            T   9 :   :   : 2 1*  :   :   :  (0X)+#
          1   #   :   : * :  0:  (X+)                :   :   : 2 1*  :   :   :  (0X)+#
I      10 1   :#  :   : * :  0:  (X+)            I  10 :   :   : 2 1*  :   :   :  (0X)+#
          1   :#  :   : * :  0:  (X+)                :   :   : 10X*  :   :   :  (+#)
M      11 1   :#  :   : * :  0:  (X+)            M  11 :   :   : 10:2  :   :   :  (+#)X*
          1   :#  :   : * :  0:  (X+)                :   :   : 10X*  :   :   :  (+#)
E      12 1   : #  :   :*  :  0:  (X+)            E  12 :   :   : 10:2  :   :   :  (+#)X*
          1   : #  :   : * :  0:  (X+)                :   :   : 10X*  :   :   :  (+#)
       13 1   : #  :   :*  :  0:  (X+)              13 :   :   : 1 02  :   :   :  (+#)X*
```

FIGURE 4–21
TIME HISTORY PROFILES – CLINICAL CARD

plotted as point 1 and those symbols outside of the parentheses are plotted as point 2 for each time segment. Channels 1 through 5 are E. coli resistant to ampicillin and sensitive to Nalidixic acid, Trimethoprim-Sulfa, and Nitrofurantoin. Threshold for E. coli is set at 50%. Channels 56 through 60 are enumeration media and since all are positive at 13 hours, the concentration would be greater than 10^5 per ml.

4.3 RELIABILITY

Topics discussed under this heading include (1) SRCLD performance, (2) media preparation, and (3) media performance.

4.3.1 SRCLD Performance - The number of SRCLDs discarded prior to the filling process was approximately 10%. This initial discard rate was due to leaks at the base of the SRCLD. Additionally, early tests were made using a total volume of 5 ml to fill the main (55) wells of the Clinical Card. This volume was not sufficient to insure complete fill in some instances. Subsequently, a total volume of 5.5 ml was used for the rehydration of these wells. To achieve a final volume of 5.5 ml, we used 5.0 ml of the 0.5% NaCl and 0.5 ml of the urine sample. The enumeration channels were filled from a second SRCLD containing a total volume of 5 ml of fluid.

4.3.2 Media Preparation - Experience gained from over one hundred commercially prepared lots of media indicate a direct correlation between Card performance and the following factors: (1) lot to lot variation of raw materials, (2) preparation procedure of media, (3) freeze dryer cycle, (4) low humidity required for Card processing, and (5) moisture resistant packaging.

Lot to lot variation of raw materials often influences performance of the final media. An example resides with the various peptones utilized in the formulations. Although well known major suppliers are utilized in obtaining these peptones, subtle differences are shown in media performance when a formulation is prepared with two different lots of the same peptone. These differences are exhibited in several ways in final performance such as slower growth of the desired organism group or false positive reactions from competing organism groups. For these reasons, large lots of raw materials are purchased, enabling production to proceed relatively

uninterrupted for long periods of time. When new lots of raw materials are required, a pretesting quality control procedure is employed to assure a smooth transition.

Preparation procedure for the selective media plays a relatively important role in assuring a reliable product. Preparation procedure is defined in several terms and includes sequence of preparation whereby ingredients are added in a predetermined manner to eliminate salting out and precipitation. Preparation procedure must be identical for each lot of Cards in order to eliminate lot to lot variation of performance.

The freeze drying cycle is of major importance in obtaining a dry product that insures shelf stability. The prefreezing rate determines crystalline structure and therefore ability of water molecules to be removed. The difference in temperature between the product and condenser determines the direction of movement of water vapor molecules. As temperature increases, the water is removed at a faster rate. The eutectic point(s) of each medium determines the primary drying cycle. Lowest eutectic determines program or cycle which must be used for entire Card. Eutectic is a function of total dissolved ionizable salts. The secondary cycle also determines whether an acceptable product is obtained; if heat is not added at the proper rate, the ΔT between product and condenser will be too small and the product will "melt back" resulting in a wet appearance.

A low humidity environment is required following removal of the product from the freeze-dryer. This environment must be maintained during the top-taping and packaging processes. A moisture-resistant pouch is required to maintain a shelf-stable product. It has been demonstrated that partial rehydration results in a dramatic decrease in performance of some selective media.

4.3.3 Media Performance - Performance of each MLM medium in recent clinical and seeded tests will be discussed under this heading.

E. coli Medium - Five false positive results were observed in 171 challenges In all cases, the positives were reported when C. freundii was present in the sample. Overall correlation was 96% for this broth.

Klebsiella-Enterobacter Medium - One false positive and four false negatives were observed in this medium in 171 challenges. Overall correlation was 97%.

C. freundii Medium - Two false negatives were observed in 171 challenges; the MLM correctly identified 7 of 9 true positives in clinical samples. The two unidentified samples failed to reach the preset threshold within the 13 hour incubation cycle. Overall correlation was 98%.

Proteus sp. Medium - No false positives were recorded but five false negatives were observed. In three of the five cases, Proteus was present in numbers of $\geq 10^8$/ml and the color reaction occurred before the Card was placed on the MLM. Consequently, the instrument was unable to detect this "instant positive" reaction, although it could be observed as a change from green to blue. Overall correlation was 79%.

P. aeruginosa Medium - The MLM failed to detect five of fourteen true positives within the 13 hour time frame. Two false positives were observed in the 171 recent challenges. Overall correlation was 96%.

Serratia Medium - One false positive and two false negative results were obtained in 171 challenges. Overall correlation was 99%.

S. aureus Medium - Seven false positives were observed with this medium; the majority (5) were due to large numbers of group D enterococcus. Subsequent investigation determined that new lots of several broth ingredients had been used in most recently prepared Cards. It was determined that false positives were directly related to the changes in raw materials. Overall correlation was 95%.

Group D Enterococcus Medium - No false positives and one false negative were recorded for 171 trials resulting in a 99% correlation.

Acinetobacter-Herellea - This medium failed to support growth of five different seeded strains of organisms tested.

Group A Beta Strep Medium - Although initial studies with this broth indicated promising results, subsequent tests in the full-up Card resulted in five false positives and three false negatives. Further work is required with this medium.

Yeast Medium - Two false negative results were noted. In both cases, the organisms were present at borderline or less than 10^5CFU/ml and did not reach threshold within the 13 hour incubation cycle.

Positive Control Medium - This medium failed to detect nine of 147 true positives for an overall correlation of 95%. Five of the nine samples contained $\leq 10^3$ organisms/ml.

Enumeration Medium - One-third of the 12 reported false negative results were due to improper filling of the enumeration wells. Overall correlation to standard plate count methods was 92%.

5.0 DISCUSSION

While some aspects of the program have inseparable biological and engineering ramifications, for purposes of discussion an attempt has been made to separate biological and engineering accomplishments and problems.

5.1 BIOLOGICAL

The task of this contract was to develop an automated microbial detection, identification, and susceptibility testing device that would be applicable to NASA specifications and use. Accomplishments in meeting this overall task were (1) development and reliability testing of the MLM selective culture media, (2) development and reliability of the MLM instrumentation, (3) demonstration of high volume sample capability, (4) demonstration of storage and return capability for positive cultures, and (5) demonstration of production of disposables.

Development of MLM selective media has been a long and tedious task and the path is littered with rejected formulations. The overall reliability of the MLM selective culture media is competitive with standard microbiological media and in many cases has been observed to exceed standard media. The MLM as a clinical instrument has been shown to give a high 90's overall score for agreement with standard tests. Data sufficient to establish the reliability of the MLM antimicrobial susceptibility testing capability have been collected and presented. Overall results have shown very good correlation to standard Kirby-Bauer susceptibility results.

Over 400,000 data points and 11,250 graphic data displays were collected with the MLM high volume carousel. Depending upon specimen source (throat, urine or feces) and MLM culture medium, the final analysis score was 92 to 100 percent agreement with standard microbiological tests. These data were summarized previously in Table 4-26.

There were numerous problems peculiar to the MLM system that are best illustrated by reviewing the developmental difficulties encountered. The data presented in this report were acquired over a period of several years; this was due to the necessity for developing and evaluating new untested manufacturing skills and methods before tests could be designed for comparison on the MLM with conventional bacteriological methods. For example, the optimum amount of each selective broth to be placed in the Card wells before lyophilization was difficult to determine because of the miniaturization involved. Accuracy of dispensing the media also presented

a problem ultimately solved by utilizing very accurate microliter syringes. Since up to 55 selective broth-antibiotic concentrations were prepared and placed in the Card simultaneously, the chance for error in compounding the broths or properly dispensing them was considerable.

Additionally, many early failures were associated with freeze-drying profiles. Due to the many products involved it was difficult to determine the best freeze-drying cycle that would work for all products. One medium, staph, has a very low eutectic point, about -35°C, so the freeze drying cycle has to start at a very low temperature to prevent melt-back. Another problem was the addition of heat either too early in the cycle or at too fast a rate. This resulted in pressure build up due to gas expansion in the media and caused media to be displaced from the well and deposited on the face of the Card. The fact that the media was in a plastic Card with the mass of plastic much greater than that of the media caused a further problem. The plastic is a very poor conductor of heat and, therefore, added to the inefficiencies of the freeze drying cycle. Eventually all these problems were solved.

Expanded testing, especially with polymicrobic specimens, revealed that media formulations gave acceptable results; some microbial combinations, however, required changes in media formulations or in computer instructions. For example, Klebsiella, when present in large numbers, will begin to grow in the E. coli broth before they ultimately become retarded by the selective inhibitors. The computer, therefore, had to be "instructed" to ignore small changes in light attenuation caused by Klebsiella but to respond to the more significant optical changes induced by E. coli.

Further testing showed that the MLM Card can serve as a reservoir for storage of positive cultures for routine microbiological evaluation upon return to the laboratory. Test results have demonstrated that most organisms can be successfully retrieved for culture following frozen storage in their selective media. The entire MLM Card can be placed in frozen storage following completion of incubation and recording of results.

The concept of automation and computerization of microbiological testing to detect, identify, and enumerate organisms from polymicrobic samples is a departure from the concepts involved in the currently used and accepted pure culture techniques

of the laboratories. The MLM has successfully combined the principle of utilizing specific compounds that selectively favor growth of specific groups or species of bacteria with automated and computerized instrumentation.

5.2 ENGINEERING DISCUSSION

5.2.1 Hardware Discussion - The engineering problem of designing, developing and fabricating a system to detect and identify microorganisms under null-g and one-g conditions was divided into four areas. Area one is the media holder in which detection occurs and resulted in two items: a Clinical Card and an Environmental Card. Area two is a method for loading the media holder with inoculum and resulted in the Sample Loading System and its SRCLD. Area three is an automated instrument to monitor the inoculated media holder and report results. Engineering effort in this area produced the Microbial Load Monitor instrument capable of incubating and reading up to five Cards. A microprocessor and related memory allows hands off determination of Card results. Area four is a method for preparing the media holder with the proper types and amounts of media. Engineering in this area produced the Media Pumping System and the Card Taper.

The problem statement identifies the media holder in some manner in all four engineering areas. The implication is that a change in the media holder will have some impact on the engineering performed for all four areas. In fact this impact occurs in more than one way. The size of the media holder (Card) determines the size of the incubating/reading head, affects the size of the loading device (SRCLD) and affects the dimensions of the Media Pumping System and Card Taper. The number and/or size of the media wells has an impact on the volume of diluent fluid needed in the loading device, on the optical characteristics of the reading head, and on the number and size of pumps used in metering media into the holder. Even the shape of the well and the optical characteristics of the media holder material affect the resulting detected signal. Tape covering the well, if stressed, can cause a lensing effect with the media/inoculum. Over a period of time when the stress is relieved, a change in detected signal will occur.

5.2.1.1 Major Hardware Engineering Problems - One major engineering problem throughout the majority of the Microbial Load Monitor System development was the minimizing of bubble effects on the detected signal. This was approached in a

variety of ways. Overflow wells or areas for unevacuated air to go have remained in most designs. Multiple wells of the same media for majority voting was considered and given some development and testing. Another method of majority voting from multiple detectors in the same well was also considered.

Each method of majority voting had its own drawbacks. Multiple detection wells increased the amount of diluent fluid needed. Multiple detectors in the same well and voting to exclude bubble shadows requires close matching of phototransistor characteristics. Adjacent phototransistors on the same processed silicon wafer would fit but would greatly increase the electronics cost. If one failed all phototransistors for that well would have to be replaced. With sixty wells per head and five heads, this would greatly increase the complexity of the MLM.

The solution to the bubble problem was in the design of the null-g Sample Loading System. Bubbles were found to be partially due to inadequate vacuum and to stressing of the tape covering the well. The tape stress introduced a negative pressure in the well and led to bubble formation during incubation as the stress was relieved. The null-g design evacuated the whole Card inside and out thereby producing no differential pressure and no tape stress. The air in the Card is not evacuated through the diluent fluid so not even its weight or vapor pressure limits the internal vacuum of the Card. Bubbles are rarely a matter of concern with the null-g Sample Loading System.

The major engineering problem on the instrument was the electronic array. Since there was no readily available array that had the electrical speed, light to electrical current gain, close mechanical alignment, and spacing in a visible red (655 nm) wave length we had to fabricate one. Fabrication difficulties with the phototransistor dies led to rework of many arrays and replacement of defective phototransistors. Double multiplexing was also needed to reduce power consumption and interconnection complexity.

The arrays after initial burn-in operation time are reliable and being solid state devices should continue to operate reliability for many thousands of hours. Microprocessor controlled calibration will adjust the drive to the LEDs for any long term changes in any portion of the electrical/optical detection path of the MLM.

5.2.1.2 Minor Hardware Engineering Problems - Minor hardware engineering problems are those which prevent the MLM from operating as designed 100% of the time. Most of these are adjustments or respecification of MLM items. Included in this category are the previously mentioned, Section 4.2.2.2, SRCLD driver magnets, needle length and retaining pin hole position.

Reprogramming of MLM programs were necessary in some cases to prevent hardware problems. In one case, the hardware time clock would be read as zero hours and zero minutes which should only occur at midnight. The clock software was changed to include a comparison of recently read time to a stored previously read time. The recently read time can be rejected (read again) or accepted and used based on the comparison.

In another case, Time History Profiles could not be plotted because of tape recorder errors. It was determined that this information could still be of value even though there would be some inaccuracies. Changes were required in the magnetic tape recorder subroutines so more software checks were written for start of record, record length and inter-record gap in addition to the normal items, record type (calibration or data), head number and checksum word computation. These checks allow continuation of plotting Time History Profiles with some data loss, though the plotted data in this case may not accurately reflect the real results provided by the instrument. Because of possible inaccuracies in plotting with data losses, plotting was only allowed for troubleshooting or for verification purposes. The troubleshooting routine EXIT is used to bypass certain PLOT program instructions to accomplish this.

5.2.2 Software Discussion - The strength of the Microbial Load Monitor Instrument is in the ability to change its function, i.e., adapt to other media, Card types, reading intervals, and incubation times. Since the whole program resides on one 8K PROM board the quickest way of changing programs would be to replace the existing printed wiring board with another one holding a different program.

Noise which could really bother a synchronous system can be minimized by averaging data values, checking a read value with a previous value (as was done with the time clock) to prevent errors, and incorporating time delays to verify signal

occurrences. True power interrupts can be verified by checking an input signal for duration of the occurrence. If it is a spike (doesn't affect normal voltage) then it will not last and can be rejected.

Other data rates for serial (teletype) or parallel (magnetic tape recorder) devices can be implemented by software changes. Additional data may also be stored or printed by additional software subroutines. The limitations are 8K of PROM space available and printing (TTY) or magnetic tape writing time available between reading each head.

5.2.2.1 Major Software Problems - One major software problem was defining how much control should be given to the operator of the instrument. The most versatile instrument would allow the operator to set up initialization parameters such as reading interval but would require more extensive programming and intimate knowledge by the operator of the software program. This would limit its use to a select few. Instead, as the instrument developed, checks were incorporated to prevent deviations by the operator from the preselected steps necessary for the Microbial Load Monitor's proper operation.

A second software problem occurred midway in the development of the MLM program and recurred just before the instrument was to be delivered. The IMP-16C Microprocessor has a hardware stack with only 16 locations for storing temporary values (data from one of the four registers, or return addresses when using subroutines). This limited hardware stack led to the development of a software stack for storing register values.

The software stack and related changes seemed sufficient until near the end of program development when the hardware stack limitations recurred. The problem this time was eventually traced to an unmodified subroutine which handled transmission of information to and from the teletype. It stored a large number of data words on the hardware stack. These were changed to storage locations in RAM to correct the problem.

5.2.2.2 Minor Software Problems - The instruction set of the IMP-16C resulted in a few minor problems when converting from low address RAM to high address PROM. The first was in subroutine jumps which in low address RAM could be made indirectly

through a low address. When put in PROM this type address would be located in the upper memory and could not be used. Two word locations in upper memory were used as intermediate steps for subroutine implied jumps (i.e., subroutine jumps were made to these locations which then made normal indirect jumps to the actual subroutine location). Approximately 64 jumps can be handled by this method but not all are used. By using this approach (versus individual indirect jumps) portions (which fit in two PROMs) of the program can be changed without having to work with the whole program and also reduces program length.

The second minor problem was the inability of the byte instruction to load or store in upper memory (32K to 64K) address where PROMs are located. Data tables which were accessed as data bytes (instead of words) had to be changed to require a whole word storage. Two tables included are media threshold values (percents) and media type (0 - no media, 1 media - no antibiotics, 2 media - with antibiotics). Thus 60 words are required for clinical media types instead of 30 words (60 bytes).

6.0 FUTURE RECOMMENDATIONS

The first and most obvious recommendation for future use of the MLM is to demonstrate its effectiveness to conduct simple automated microbiological analyses in the microgravity space environment. Although the system was conceived and designed for microgravity operation, as well as for simplified use, only an actual flight demonstration will determine its true utility in the space environment.

The inherent capabilities of the MLM for inflight usage provides for the first time the ability to conduct real time inflight growth studies which can measure both bacterial growth and metabolic rates. As designed, the system can detect and enumerate microorganisms, both bacteria and fungi, which may be of potential harm to space travelers. These microorganisms include those which can cause acute illness as well as those opportunistic microorganisms which may pose a threat to susceptible individuals.

Based on traditional light absorption principles, but highly miniaturized and computer aided, the technology has wide applications in other areas of clinical microbiology, hematology and clinical chemistry. As a research tool for conducting microgravity studies on bacterial and animal cell growth rates and metabolic behavior in the microgravity, there are no equivalent systems. Even more basic studies such as those on enzyme or other chemical kinetic studies in isolated systems would be conducted in controlled environment to measure differences in molecular-molecular interactions as affected by gravity versus nongravity situations.

These capabilities, with successful demonstration of the system during a spaceflight experiment, could provide the basis for a long needed piece of Common Operating Research Equipment (CORE) for applied and basic research studies with a variety of biological and chemical systems.

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